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Adoptive Cell Therapies: A Cell Culture Media Perspective

Purpose-driven Media Design for ACT Development

IMMUNOTHERAPY BACKGROUND

Adoptive cell therapies (ACTs; also known as cellular immunotherapies) are a treatment modality with tremendous potential for treating a myriad of diseases ranging from cancer, to infectious diseases, autoimmune disorders, as well as transplant related complications (Dwarshuis et al, 2017).

Two such breakthrough treatments recently approved by the FDA are Kymriah (Novartis) and Yescarta (Gilead/Kite Pharma); chimeric antigen receptor (CAR) T cell therapies for the treatment of acute lymphoblastic leukemia and large B-cell lymphoma, which have demonstrated incredible success in clinical trials with remission rates as high as over 80%.

Their success has garnered growing interest and enthusiasm from the pharmaceutical and investment communities, forging an important path in defining success criteria for future ACTs and identifying the gaps that still remain, including scale-up and harmonization of manufacturing and regulatory standards.

Importantly, much of the pharmaceutical regulatory guidelines and manufacturing considerations currently in place for ACTs find their source from the 30+ years of groundwork laid by the biologics industry (Sargent 2016).

Currently—though there are 2953 active, recruiting, or completed immunotherapies on clinicaltrials.gov (as of January 2020)—ACTs remain “boutique therapies” applied to a relatively small number of patients in comparison to traditional pharmaceuticals. This is due to the complex and labor-intensive manufacturing processes currently required and the high associated costs (Dai et al, 2019).

Living cells and tissues are inherently dynamic, adding a fundamental complexity to the manufacturing and scale-up process not present in most non-biologic therapies (NAS 2017).

Without the ability to reproducibly manufacture large numbers of high-quality cells at low cost, the clinical potential of ACTs cannot be fully realized.

CURRENT CHALLENGES

According to Aijaz et al, 2018, the conversion rate of cell-therapies from phase III to FDA approval is considerably lower than mature pharmaceutical drug classes; 14.3% and 48.7%, respectively.

Generally, the mechanism of action (MOA) of ACTs is often poorly understood due to their inherent complexity, presenting a major challenge in identifying critical quality attributes (CQAs) which the FDA defines as: physical, chemical, biological, or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality (FDA Q8(R2)).

Without a clear MOA, defining and measuring properties that can ensure functional quality (potency), purity, and reproducibility of the final product will likely be infeasible (Dwarshuis et al, 2017).

Potency is critical to determine early on as it will be used for important decisions on product-lot release, shelf life, comparability between products manufactured within or between sites, and validation of clinical preparation (Aijaz et al, 2018).

Without well-defined CQAs, critical process parameters (CPPs) are difficult to determine, which are necessary for cell quality and consistency across batches and facilities (Dwarshuis et al, 2017).

The FDA defines CPPs as a process parameter whose variability has an impact on a CQA and, therefore, should be monitored or controlled to ensure the process produces the desired quality (FDA Q8(R2)). Important CPPs include culture conditions and apparatus, duration, and media compositions (Dwarshuis et al, 2017).

These hurdles ultimately present significant challenges in adhering to Quality-by-Design (QbD) principles required for well-optimized processes that would enable high-quality, large-scale production of therapeutic cells (Dwarshuis et al, 2017).

The FDA defines QbD as a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound scientific and quality risk management FDA Q8(R2)).

continued

In other words, QbD is achieved by clearly defining product quality attributes as well as features and parameters with direct influence on product safety and efficacy, and developing a design space able to quantify effects of parameter variability on these quality attributes (Dai et al, 2019; Lipsitz et al, 2016).

Current Good Manufacturing Practice (cGMP) regulations are enforced by regulatory bodies (eg, FDA) to ensure that methods, facilities, and controls used in manufacturing, processing, and packaging of a drug product result in a product that is safe for use with ingredients and strength it claims to have. cGMP regulations, when viewed in their entirety, incorporate the concept of QbD (FDA Quality Systems Approach).

In the context of utilizing living cells as therapeutic agents, important technical and logistical aspects that need to be considered are cell expansion, separation, automation, and preservation, as well as the need for a robust supply chain management strategy and shipping/packaging techniques (Dwarshuis et al, 2017).

Though a product may demonstrate safety and efficacy, it can still fail to meet cGMP requirements, delaying approval until all manufacturing concerns are addressed.

A recent example is Enzyvant's RVT-802, a tissue-based therapy in development for congenital athymia. Though RVT-802 was granted Breakthrough Therapy designation, Rare Pediatric Disease designation, Orphan Drug designation, and Regenerative Medicine Advanced Therapy (RMAT) designation, the US Food and Drug Administration (FDA) unfortunately had to reject the therapy based on manufacturing concerns (Bioprocessintl).

For the purposes of this review, we approach these development- and production-related challenges from a cell culture media perspective and highlight its importance as a critical ancillary material throughout the development, manufacturing, and scale-up of ACTs.

Consideration of cell culture media early on in the process can help to save valuable time, money, and resources, especially when transitioning into the clinical trial phase and further scale-up. A cell culture media that is well-defined and sourced from cGMP compliant components is a critical factor to take into consideration as early on as possible.

CELL CULTURE MEDIA BACKGROUND

Cell culture media is generally a complex mixture comprised of varying types and amounts of amino acids, hormones, growth factors, proteins, vitamins, trace elements, and lipids, along with attachment factors, shear force protectors, protease inhibitors, protein hydrolysates, and antibiotics as needed.

Animal derived serum (human or non-human) is the most commonly used cell culture supplement that contains many of the essential components necessary for cell growth and maintenance, though highly undefined in composition (Valk et al, 2010). Serum components, such as serum albumin, etc. are routinely used in the formulation of serum-free medium in a desire to move toward more highly defined media.

TYPES OF CELL CULTURE MEDIA

- **Serum-containing media (SCM)** is the least defined and relies on serum to supply essential factors and is commonly used for most cell culture applications.
- **Serum-free media (SFM)** represents a broad category of media that does not require serum supplementation and can range from defined to undefined based on its composition.
- **Protein-free media** do not contain any proteins but can contain undefined peptides from plant hydrolysates which are undefined in composition.
- **Chemically-defined media (CDM)** do not contain any undefined animal-derived raw materials and are free of any animal-derived components, including human plasma or human blood-derived components. All raw materials are defined and their exact concentrations are known, which can include recombinant proteins.
- **Xeno-free media (XFM)** do not contain components of animal origin but can be supplemented with components of human origin (eg, human serum albumin, platelet lysate, etc). Protein hydrolysates or plant extract (all derived from the same species) may also be added.
- **Animal-derived component-free media (ACFM)** are manufactured with raw materials that do not contain any animal- or human-derived products to the tertiary level and are manufactured using equipment that does not come in contact with any animal or human products. The raw material handling, storage, and final product manufacture and packaging are performed in dedicated human- and animal-component free facilities and equipment.

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UNDEFINED COMPONENTS

Since the American embryologist, Ross Granville Harrison, developed the first *in vitro* cell culture technique in 1907 using lymph medium (Harrison 1907), animal blood-derived fractions and components remain the most common supplement utilized in animal cell culture.

Currently, fetal bovine serum (FBS) is considered nearly universal and widely used across numerous cell types (including research products and cellular therapies) due to its rich content of growth factors and hormones while maintaining low levels of antibodies and gamma-globulins compared to other animal sera (Shah 1999; Barnes 1980).

A recent assessment by Mendicino et al, 2014, found that over 80% of regulatory submissions for mesenchymal stem cell-based products describe the use of FBS during manufacturing. Similar statistics are believed to be applicable across a wide range of cell therapy products, including ACTs (Minonzio et al, 2014).

However, the largely undefined and variable composition of FBS, as well as other animal-derived components, present significant regulatory, production, and supply chain concerns which are discussed in further detail below.

REGULATORY CONSIDERATIONS

Though animal serum and serum-derived components, including FBS, can be utilized in the clinical translation and production process, their use is accompanied by a cogent risk of transferring adventitious agents as well as the potential for inducing immunogenicity (Minonzio et al, 2014).

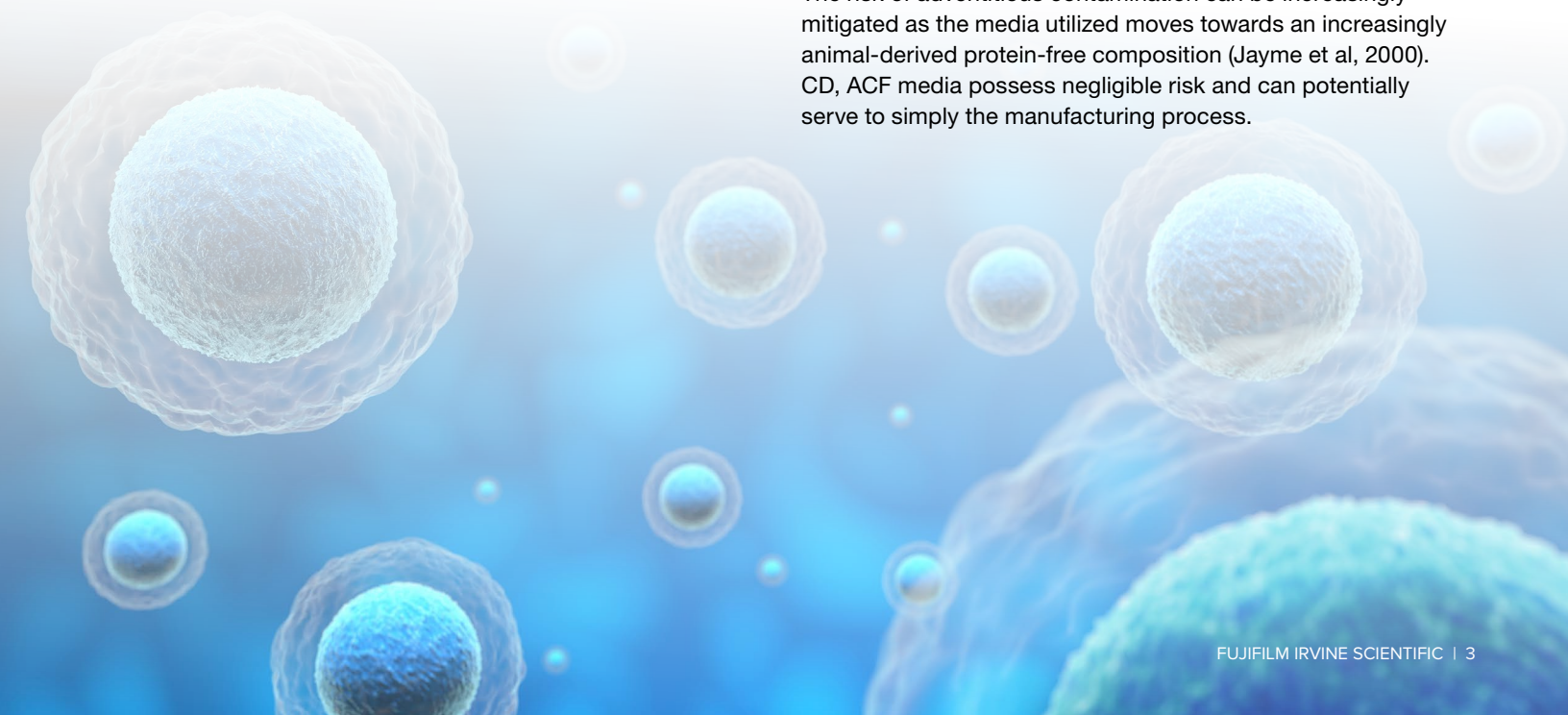
As such, important yet costly and time-consuming requirements have been set in place by regulatory bodies (eg, FDA) to mitigate the risks, which ultimately translates to greater costs associated with ACT translation and manufacturing.

For example, in order for FBS to receive cGMP designation, it must be sourced from cattle herds raised in countries approved for import by the USDA. The health-status of the animals must be well monitored and the FBS processed under strict cGMP standards requiring each FBS lot to be traceable to the country, slaughterhouse, and herd of origin (Minonzio et al, 2014).

Additionally, lots must be tested for potential viral contamination, sterility, mycoplasma content, endotoxin levels, hemoglobin and IgG concentration, etc., as outlined in 21 CFR Part 210 & 211 (Minonzio et al, 2014). This is critically important as up to 20–50% of commercial FBS is reported to be virus-positive (Wessman 1999).

Furthermore, the FDA Code of Regulations for Biologics (21 CFR 610.15) requires animal serum levels to be under 1 ppm in the final product, regardless of when serum is used during the production process, which can necessitate additional processing steps or major changes to ensure compliance.

The risk of adventitious contamination can be increasingly mitigated as the media utilized moves towards an increasingly animal-derived protein-free composition (Jayme et al, 2000). CD, ACF media possess negligible risk and can potentially serve to simply the manufacturing process.



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PRODUCTION CONSIDERATIONS

Of the numerous constituents and complex mixture that make up serum, growth factors and other growth promoting biomolecules play an essential role in supporting cell culture. Unfortunately, considerable quantitative and qualitative variability is present in the composition of each lot (Minonzio et al, 2014). As such, the levels of key growth factors, hormones, etc. can vary widely from lot-to-lot, as well as when using different concentrations of a given lot, ultimately resulting in ACT product inconsistency.

Furthermore, supplementation of serum to basal medium already containing defined levels of key components can result in concentrations that inhibit cell growth and/or function (Poiley et al, 1978). Notably, lot-to-lot variability and the potential for "over supplementation" is also observed for other highly undefined media supplements, such as protein hydrolysates (Valk et al, 2010). Serum and other undefined media components can present a significant challenge to media optimization efforts and implementation of QbD.

To overcome inconsistency between lots, expensive and time-consuming qualification studies are required where multiple serum lots must be tested across several donor cells in order to identify the optimal lot for use during the production process (Minonzio et al, 2014). For example, each FBS lot must be screened by the end user to assure its consistency and reproducibility in terms of cell growth, potency, etc (Minonzio et al, 2014; Even et al, 2006).

The challenge is further compounded by the limited capacity to meet growing demand. For example, the global production of FBS is estimated at 500,000 L, equating to 1,000,000 bovine fetuses being required, which is not always possible (Minonzio et al, 2014; Even et al, 2006; Jochems et al, 2002). Serum-free medium can help mitigate such sustainability risks (Tan et al, 2016) and even increase cell yields in certain cases (dos Santos et al, 2011), ultimately helping to reduce cost of goods per dose (Aijaz et al, 2018).

Cell culture media can be a major cost driver from early development to cGMP manufacturing, with the latter being more likely to see significant impacts from factors such as feeding scheme, labor, waste disposal, and storage. This highlights the importance of maximizing media efficiency by minimizing total media volume required for maximal cell growth and function (potency); a concept that draws from the insights gained throughout several decades of biologics manufacturing (JRR).

Though per liter cost of media is an important factor to consider, greater focus should be placed on the cost of media per therapeutically active cells produced (media productivity) as a medium with higher per liter cost can still produce incremental cost reductions by improving cell growth and potency (JRR).

Additionally, transitioning from smaller-scale static culture to a bioreactor system that is automated and scalable is not a trivial process and will likely require a change in packaging (bottle to bag format), or even a completely different cell culture media formulation, which can result in significant delays and additional costs.

Therefore, it is highly recommended to partner early on with cGMP compliant vendors to identify the optimal cell culture media and other ancillary materials to ensure highest media productivity. Importantly, there are cell culture media vendors with expertise in numerous cell types that provide custom media services to help tailor media formulations for optimizing cell growth and potency.

With the inherent intricacies to media development and manufacturing, a rational media design approach can meet the challenge of developing a sophisticated medium that tailors to specific processes within a short amount of time (IS).

SOURCING AND SUPPLY CHAIN CONSIDERATIONS

Supply chain considerations of ancillary materials are a critical aspect of the manufacturing process often overlooked until later stages of clinical development. Supply and sourcing issues can lead to major manufacturing delays that result in valuable time lost for waiting patients. As an ACT moves into clinical development, cGMP sourcing is required for all ancillary materials to ensure product safety.

For example, the FDA will likely require cGMP sourcing in order to approve an investigational new drug (IND) application. Device or drug master files (DMFs) for each material, including cell culture media, will be reviewed alongside the IND, which provides confidential, detailed information about facilities, processes, or articles used in the manufacturing, processing, packaging, and storing of human drug products (FDA DMF).

If non-cGMP materials were utilized throughout early development or a selected vendor is faced with supply issues, it can take several months, if not longer, to resource and requalify and test the materials before production can begin (JRR). All ancillary materials need to be qualified as pertains to source, purity, identity, safety, and suitability, and the level of qualification required will vary based on the degree of risk associated with their composition and intended use (Solomon et al, 2016).

For example, CD, ACF media requires less qualification than media supplemented with serum. Though a certificate of analysis may be provided by the vendor, undefined components such as serum still require additional qualification steps due to high levels of lot-to-lot inconsistency.

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CONCLUSION

Living cells are inherently complex and highly dynamic, presenting a myriad of challenges toward the development of ACTs. A high bar has been set to ensure safety and efficacy for patients, necessitating strict regulatory requirements and guidelines regarding the manufacturing process. Among the many factors and components involved throughout, cell culture media plays a quintessential role from early development to full-scale manufacturing and should be thoroughly considered early on with scale-up in mind.

Tailoring and simplifying its composition by moving away from animal-derived and undefined components can help to reduce safety risks and lot-to-lot variability, ultimately reducing the likelihood of unexpected delays and costs. Partnering early on with an experienced and knowledgeable media supplier can ensure smoother transition throughout the key development and production milestones in successfully taking ACTs from the bench to the bedside.

FUJIFILM IRVINE SCIENTIFIC: COMPANY OVERVIEW

FUJIFILM Irvine Scientific, Inc., is a worldwide leader in the innovation and manufacture of cell culture media, reagents, and medical devices for researchers and clinicians. The company provides unrivaled service and quality to scientists working in biopharmaceuticals, cell therapy and regenerative medicine, assisted reproductive technology and cytogenetics, and industrial cell culture for the large-scale production of biotherapeutics and vaccines.

FUJIFILM Irvine Scientific adheres to both ISO and FDA regulations and operates dual cGMP manufacturing facilities in California, USA, and Tokyo, Japan, along with plans for an additional facility located in Tilburg, Netherlands by 2021.

The company’s consultative philosophy combined with expertise in cell culture and compliance provides customers with unique capabilities and support.

For over 50 years, FUJIFILM Irvine Scientific has remained uniquely flexible and focused on media while becoming a strategic global leader in media products and services. FUJIFILM Irvine Scientific, Inc. is a subsidiary of FUJIFILM Holdings America Corporation reporting to FUJIFILM Holdings Corporation.

SERVICES

Life-changing therapies are based upon the foundation of optimal cell culture media. Finding a good medium is important because it affects process performance, but there is no single medium for every application. Designing media with the end goals in mind requires a more purpose-driven, rather than random, approach that replaces guesswork with expertise.

FUJIFILM Irvine Scientific offers a diverse portfolio of advanced cell culture media solutions including media products, services, and technologies for bioprocessing targeted to meet the evolving demands of the biopharmaceutical, vaccine, and gene therapy industries.

Nimble, extremely responsive, and deeply collaborative, we work with each of our customers at every stage—from early research through commercial production—to develop personalized solutions that exceed expectations. With a culture rooted in innovation, partnership, and customer service, we dedicate our resources and expertise to helping you expedite therapies to the patients who need them most.

RATIONAL CULTURE MEDIA DESIGN

Rational Culture Media Design leverages FUJIFILM Irvine Scientific's focus on cell culture science and process knowledge to construct rational experiments that aim to reach a target medium's end goals. We aim to apply our continually growing knowledge-base to solve specific problems through hypothesis testing rather than trying many variables and seeing what sticks.

While experimentation with unknown variables is unavoidable, with a commitment to applying what we learn in a thoughtful manner, the design process becomes more efficient and better understood for future applications. This systems biology view of cell culture processes focuses on translating large amounts of data into a solution.

We understand that the choice of media is just one of many factors required to achieve a successful cell culture process. We scrutinize all of the key elements of a process in order to identify specific opportunities for improvement, whether growth rate, cell density, cell potency, or any other factors, before developing appropriate media designed to deliver what is required by your process.

Every process is different, depending on the cell type, equipment configuration and process parameters. Cell culture media should therefore be tailored appropriately to the cell type/application of interest to ensure maximum media productivity.

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MEDIA DESIGN AND OPTIMIZATION

At FUJIFILM Irvine Scientific, we provide a complete work solution for your immune or stem cells either with our or your IP formulation.

We work collaboratively with you as a partner to discover, develop, and perfect media that maximizes the potential of your cell/product performance, while delivering the service and support you expect from a leader in cell culture media.

As a leader in cell culture media manufacturing since 1970, our R&D experts understand how to help you create a custom media solution and bring it full-scale for commercialization.

MEDIA SURVEY PANEL

From the moment that you request a Media Survey Panel—through development and into bioproduction—we provide comprehensive support from our sales team, field application scientists, and scientists in R&D. Our experts personally select your Media Survey Panel, so that the development process starts with a refined, more focused level and achieves the results you need sooner.

Discovering the optimal cell culture solution begins with evaluating a curated Media Survey Panel from FUJIFILM Irvine Scientific. Each Media Survey Panel features a diverse selection of off-the-shelf, made-to-order, and cGMP-ready media samples, customized for your cell line and application, as well as the protocols necessary to maximize performance. All of our media are formulated for scalability, quality, and process consistency—this is where the development of custom media begins.

- Partner with your dedicated FUJIFILM Irvine Scientific team to access unrivaled cell culture expertise and an extensive media library based on more than 45 years of research and development.
- Selection of different media formulations developed for a wide range of cell lines, based on your capacity and application.
- Customized, comprehensive protocols to evaluate your custom media panel for improved growth, productivity, and quality.
- Panels shipped to you in as little as 10 business days—one of the fastest turnaround times in the industry.

CUSTOM MEDIA DEVELOPMENT

Custom media development services start with a promising formulation—for example from your Media Survey Panel or based on a catalog medium—and modify it for your particular needs. Support from our highly qualified technical team within R&D makes all the difference between “a medium” and “your medium.”

Once you have identified a promising medium, we develop it further via our Media Development and Optimization (MDO) Services. Depending on your available time, instrumentation, and personnel, you may proceed with either a Media Optimization Panel or Complete Service MDO option to develop your new media.

Media Optimization Panel (MOP)

Your project is conducted within your laboratory, with protocols, instructions, and the custom media necessary to perform the cell culture experiments provided by FUJIFILM Irvine Scientific. With each MOP, we design the experiments, send the custom media panel, analyze the results, and manage the timeline while you control the speed of development.

Complete Service (CS)

Your project is conducted within FUJIFILM Irvine Scientific’s laboratories to develop and deliver a cell culture media solution that meets your specific process requirements, such as improved cell growth, maximal titer, and desired product quality.

With a Complete Service MDO, we are able to deliver the most improvement in a shorter amount of time than with MOP, since all of the work is performed in-house at FUJIFILM Irvine Scientific. You may halt the optimization process at any time that satisfactory results are achieved and quality.

EXPRESS MEDIA SERVICE

With the Express Media Service (EMS), our laboratory becomes a virtual extension of your own, offering you flexible, small-scale media production, as well as accessibility to our dedicated EMS R&D team for formula review and consultation.

Our Express Media Service (EMS) provides rapid, flexible, small-scale media production to expedite time-to-market needs. Customer-developed formulas and modified classical formulas are manufactured in a non-GMP environment using cGMP-grade raw materials, in as little as 10 business days.

Express Media Service can also serve to manufacture small lots and pilot scale lots of media to test the feasibility of scale-up. Stability testing is also available upon request. Stability testing is also available upon request.

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MEDIA PRODUCTION

Custom Powder and Liquid Media Manufacturing

Uninterrupted and rapid product supply of cell culture media is critical, so FUJIFILM Irvine Scientific is continually improving to meet the challenges our customers face. In order to address these concerns, FUJIFILM Irvine Scientific has state-of-the-art powder manufacturing facilities located in both Santa Ana, California and Tokyo, Japan. Both facilities provide complete redundancy in milling technology, raw materials, processes, and quality systems.

Market requirements for filling sterile liquid media and reagents in flexible bulk packaging are driven by specific processes and applications. At FUJIFILM Irvine Scientific, we offer versatile solutions for bulk liquid packaging for basic or intricate custom requirements to meet pre-determined customer specifications (eg, static vs dynamic expansion systems).

Currently, we offer single lot sizes of up to 10,000L and the capability to fill liquid into vials, bottles, or bioprocess containers (BPC) within our multiple aseptic filling suites. We achieve lot-to-lot consistency through highly detailed manufacturing procedures, precise tolerances on all measures, and strict in-process acceptance ranges.

Raw Material Handling

Because a high-performing medium is essential to the success of our customers' programs, FUJIFILM Irvine Scientific realizes that full control from its prototyping, through scale-up or scale-down modeling, during troubleshooting, and throughout production requires the highest quality products and rapid response to customers' needs.

As media are only as pure as the least pure ingredient, our stringent Raw Material Program ensures a final product that exceeds all quality requirements. FUJIFILM Irvine Scientific achieves lot-to-lot consistency through highly detailed manufacturing procedures, precise tolerances on all measures, and strict in-process acceptance ranges.

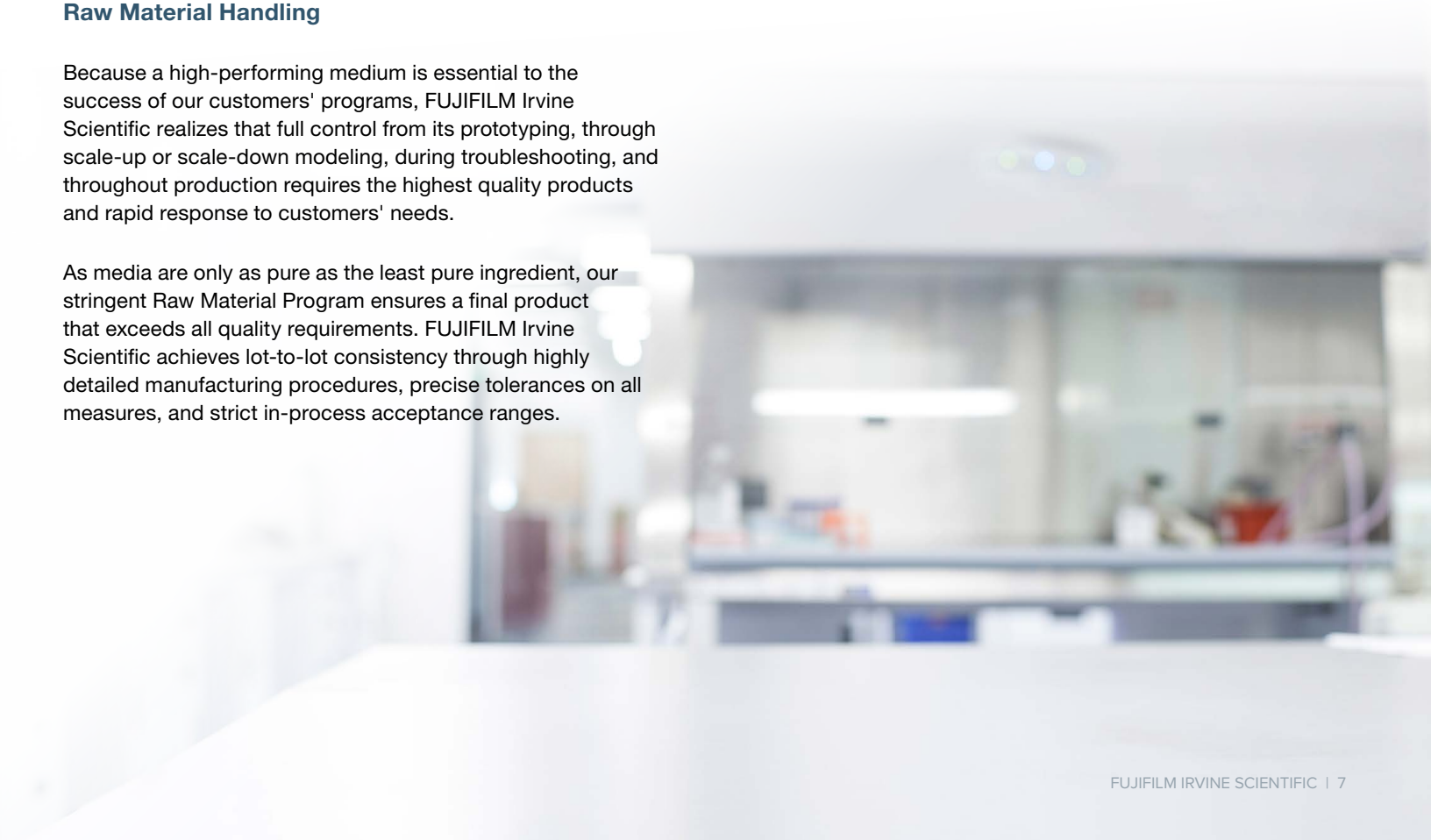
EXPEDITED SERVICE

Custom media orders are completed in just 8–10 weeks—the fastest industry turnaround time, with large-scale cGMP manufacturing of dry powder up to 7,000 kg and liquid media up to 10,000 L.

LOGISTICS

The Shipping and Logistics department at FUJIFILM Irvine Scientific is dedicated to sending our customers' products with the best packaging and shipping methods to ensure the shipments reach our customers on time and in quality condition. We take pride in how we pack and in the logistics companies with whom we ship.

Our packers are very experienced with both domestic and international shipments. We ship to the continental United States (as well as Alaska and Hawaii) and to many countries across Asia, Australia, Europe, South America, Africa, and the Middle East. We understand our routes and work closely with our logistical freight providers to ensure the highest quality is maintained.



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FUJIFILM
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