



White Paper

Advanced Granulation Technology™ (AGT™ dry media format) Culture Media

Benefits and Case Studies

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Advanced Granulation Technology™ (AGT™ dry media format) Culture Media Benefits and Case Studies

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Executive Summary:

This White Paper overviews the decision factors that end-users consider when evaluating dry powder media vs. AGT™ dry media format, and summarizes situations where AGT™ dry media format may provide specific benefits over alternatives. It offers a review of how switching can address the problems inherent in dry powder media preparation and usage. The three case studies from multiple end-users present experiences related to improved performance by using AGT™ dry media format, and the key factors that influenced decision-making.

AGT™ dry media format culture media offer significant benefits compared to other media formats, including conventional DPM; manufacturer-prepared bulk liquid media; and liquid media concentrates. AGT™ dry media format media preparation and use are simpler, less expensive, faster and more consistent. This can make AGT™ dry media format an attractive in-house platform for mammalian cell culture media applications. AGT™ dry media format media have been, and continue to be, the fastest-growing media product line from Thermo Fisher Scientific (Gibco®), the culture media market and innovation leader. Users have come to rely on AGT™ dry media format media quality and its supplier's robust supply chain.

For further information, contact your Thermo Fisher Scientific media sales representative and/or visit www.lifetechnologies.com/agt

Introduction:

Culture media are the most complex of bioprocessing essentials. Some include 50 or more different nutrient components. The most basic factor differentiating culture media is its physical state, with the conventional dry powder media (DPM) format largely controlling the processing and costs involved in producing the final liquid culture media for use in bioreactors. This White Paper discusses current problems encountered by users associated with the preparation and use of DPM. It then evaluates Advanced Granulation Technology™ (AGT™ dry media format), an alternative dry format culture media from Thermo Fisher Scientific (Gibco®/LTI). This paper then provides end-user experiences with AGT™ dry media format. AGT™ dry media format refers to both the unique process used to manufacture highly dissolvable powder granules and to the resulting mammalian cell culture media products.

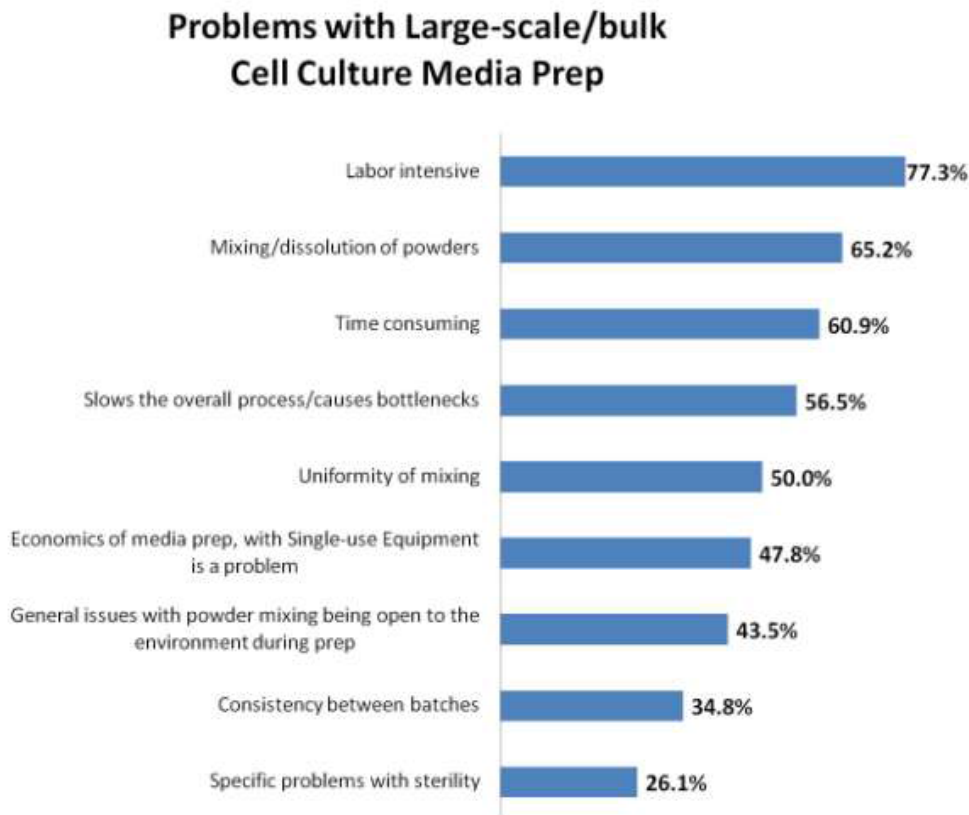
The two common culture media format options are finely ground powders (DPM) for end-user in-house hydration (mixing and dissolution) to prepare finished liquid culture media, and bulk liquid culture media purchased directly from media manufacturers. These are generally fully diluted but in some cases come as liquid concentrates. Both media formats are usually manufactured to cGMP standards. Manufacturer-prepared liquid media are generally too expensive for most uses, with the end-user having to pay for long-distance shipping of what is mostly water [Water for Injection (WFI)]. Another problem with prepared liquid media is their limited shelf life, which may be limited to days or weeks. DPM and AGT™ dry media format can be stored much longer; the product is stable for up to 2 years. Although prepared liquid media has some advantages, many end-users lack storage space for liquid, rehydrated media.

Currently, $\geq 90\%$ of culture media, particularly at any larger scales, is purchased as DPM. This powdered form is then rehydrated by end-users, in-house. Preparation of liquid culture media from powders is a core venerable part of bioprocessing. Most bioprocessing facilities are experienced with preparation of liquid culture media from powders. These bioprocessing facilities are currently designed to handle powders in dedicated areas for DPM storage, preparation/hydration, liquid media holding/storage, and sterile filtration prior to filling bioreactors. Large-scale facilities can devote as much as 20% of production space to DPM media and its preparation, with media having a major impact on operations and related costs.

Problems Users Face with Dry Powdered Media (DPM)

Market research by BioPlan Associates, Inc. documents many end-user problems and complaints regarding preparation of liquid media from ground conventional DPM powders [1]. Over 75% of those surveyed cited “Labor intensive” as the number 1 problem with preparation of liquid media from DPM. This was followed by “mixing/dilution of powders” (65.2%); “time consuming” (60.9%); “slows the overall process/causes bottlenecks” (56.5%); and “uniformity of mixing (50.0%). All of these problems, each cited by a majority of respondents, are substantially alleviated using AGT™ dry media format media. Labor, time required, and problems with mixing were the most common problems/complaints.

Fig 1. Percentage of Respondents' Indicating Large-scale Cell Culture Media Preparation from DPM to be a "Significant" (5) or Greater Problem



Source: BioPlan Associates, Inc., research conducted in December 2013

Many end-users reported serious problems in preparing liquid media from DPM, including:

- Powders clumping
- Floating
- Problems associated with less than optimal solubility;
- Powders going into the air during transfers, causing dusts requiring monitoring and operators wear protective equipment;
- The ‘same’ DPM manufactured at different scales being different, e.g., use of different milling and blending, and behaving differently in cell culture; and
- Too many different required additions (basal media supplements), often a dozen or more, with each supplement increasing contamination risks and adding to time and quality control costs.

Users also report generally having to adjust pH and/or osmolality of liquid media prepared from DPM, requiring addition of hazardous acids or bases. Essentially, all these problems with DPM preparation increase in magnitude as scales go up.

A common complaint was inconsistency in culture media performance associated with variations in preparation from DPM. This includes the same media when prepared by end-users at different scales, i.e., problems with scalability. Conventional DPM mixing requires experience to deal with the potential for variability. It can be a challenge for end-users who lack specialized equipment and expertise, to consistently prepare liquid media from DPM. AGT™ dry media format, with its improved rapid dissolution, has been shown to largely resolve these problems.

Users also confirmed that culture media formats, e.g., DPM vs. prepared bulk liquid media, are usually changed during a product’s development. For example, R&D and process development at small scales typically use liquid culture media, such as 1 L bottles. But most facilities switch to using DPM, usually a fully custom product (adding costs), as bioprocessing scales increase, particularly before the start of Phase III and commercial manufacture. Dry media, both DPM and AGT™ dry media format, are significantly cheaper than finished liquid media from media manufacturers, with its prohibitive shipping costs in addition to the added costs for liquid preparation, difficulties in storing large volumes of liquid, and other quality control issues[2].

Users Actively Seek Solutions for Media Preparation Problems

Trends in bioprocessing are being driven by the need for efficiency, flexibility, quality, consistency, and cost reductions in manufacturing processes. To remain competitive, better technologies are needed that cut down on time-to-market and streamline product development, testing and eventual commercial manufacturing. End-users want flexibility in their bioprocessing supplies. Users also increasingly prefer to adopt facility- or company-standardized platform technologies and products vs. using a variety and more costly custom-manufactured products.

Many users seeking these goals have reconsidered and upgraded their bioprocessing in recent years. This includes rather widespread adoption of single-use mixers and other equipment for media preparation; and adoption of advanced culture media, such as AGT™ dry media format. The product was launched 10 years ago, and is now well established with a solid track record. It is being utilized by over 300 bioprocessing facilities at all scales, including those manufacturing multiple commercial products.

In fact, according to comment from end users, AGT™ dry media format is well-suited for the “facilities of the future” where users are engineering multi-product, multi-scale and fully single-use facilities. From the BioPlan Associates Annual Report of Biopharmaceutical Manufacturing, Mammalian cell culture yields have increased an order of magnitude in recent decades, e.g., from 0.1-0.3 g/L to now over 3 g/L (newer processes are averaging 3.21 g/L at clinical scale and at 2.56 g/L at commercial-scale)[2]. As a result of this and other advances, there has been an order of magnitude reduction in bioprocessing scales, e.g., with legacy monoclonal antibody manufacture generally involving use of multiple $\geq 10,000$ L bioreactors, while current technology readily supports manufacture of the same amount of products using much smaller, e.g., $\leq 2,000$ L, bioreactors, increasingly single-use (we note that stainless steel bioreactors still dominating use for commercial manufacturing) [3].

AGT™ Dry Media Format Media Promotes Switching From DPM

Users increasingly report seeking to avoid problems with culture media variability, including avoiding media containing hard-to-characterize, often inconsistent, complex

organic mixtures, such as plant hydrolysates. AGT™ dry media format, which is fully chemically-defined and free of animal products and complex natural product mixtures, avoids these problems. This includes avoiding concerns for media as a source for prion (BSE/TSE) contamination of products and animal virus facility-wide contamination.

As discussed below, AGT™ dry media format media benefits have influenced DPM users to initiate evaluations. Benefits being assessed include its consistency as an off-the-shelf (but also customizable) product that is simple and quicker to prepare, that increases flexibility, and is more cost-effective than conventional DPM or bulk prepared liquid media. Users report (see later section) that the conversion to AGT™ dry media format has helped them address many of the problems noted above.

Overview of the DPM vs. AGT™ Dry Media Format Technology

AGT™ dry media format is manufactured using a unique patented process that provides many improvements compared to conventional DPM. The AGT™ dry media format culture media are single component, fully chemically-defined, particulate dust-free, rapidly soluble powders for mammalian cell culture. The technology enables the manufacture of advanced dry media with many complex media formulations, including those developed by end-users.

AGT™ dry media format media are 'single-component' in the sense that the component ground powders composed of different nutrients are homogenous. During mixing of component ground nutrient powders, the powders are moistened, forming agglomerating and congealing to larger granules or clusters composed of many ground powder particulates fused together. These larger congealed particles provide advantages including much more rapid dissolution when added to mixers and no formation of dusts posing inhalation and ocular exposure hazards.

AGT™ dry media format media are manufactured using patented fluid(ized) bed granulation technology. This enabled the product to be the

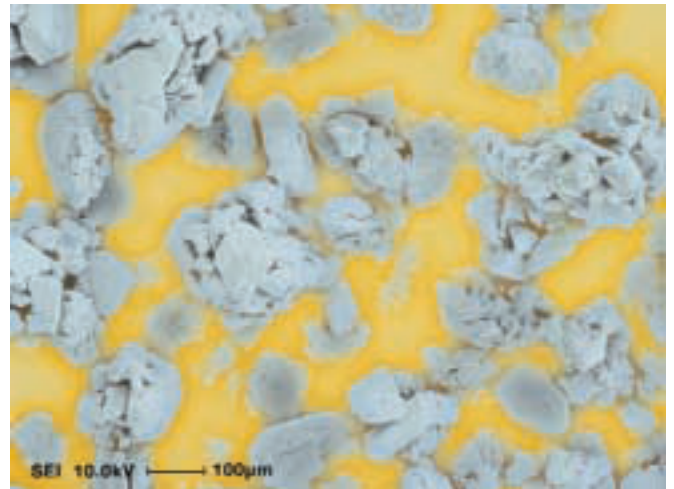
first commercial, complex, serum-free, protein-free, and chemically-defined media in a dry format. Dry component nutrient powders are suspended using a continuous column of conditioned air flowing up from underneath (see 250 kg/14,000 L capacity equipment in Fig. 1). The free-flowing powder particles are sprayed with a fine mist of aqueous solutions, with this evenly distributing many trace components. As the water dries, larger particles form from surface fusion of partially-dissolved powders into porous, larger, free-flowing, highly water-soluble granules ready for rapid hydration and use (see Fig. 2). The gentle, controlled, minimal-shear environment of the fluidized bed does not adversely affect the various nutrient components being mixed and fused together. After granule formation, AGT™ dry media format granules are further milled and sized in a Fitz mill, and blended in drum ranging from 50-1,500 kg and very large blenders handling up to 6,000 kg. AGT™ dry media format, as exemplified by CD CHO AGT™ dry media format, is typically used at a concentration of 24.3 g/L, with each kg providing ~41 L of liquid media and 6,000 kg providing nearly 250,000 L, enough for manufacture of up to 25 mAb production lots each using 10,000 L of media.

The AGT™ dry media format manufacturing process is fully scalable, producing consistent media irrespective of lot/batch size, which can range from 2 to 6,000 kg. HPLC and other analyses of AGT™ dry media format lots/batches show that biochemical constituents are homogeneously distributed throughout the granules, and that nutrient levels are comparable to conventional formats; in addition to comparable cell culture performance compared to relevant reference media products.

Fig. 2: Fluidized bed granulation equipment



Fig. 3: Microscopic view of AGT™ dry media format large, porous, highly water-soluble granules



AGT™ dry media format comes in a variety of stock/catalog formulations, each optimized for specific applications:

- CD CHO AGT™ dry media format Medium
- CD OptiCHO™ AGT™ dry media format Medium
- CD FortiCHO™ AGT™ dry media format Medium
- VP-SFM AGT™ dry media format Medium
- OptiPro AGT™ dry media format Custom Medium
- CD Hybridoma AGT™ dry media format Medium
- CHO CD EfficientFeed™ A, B & C AGT™ dry media format Nutrient Supplement
- CHO CD EfficientFeed™ A+, B+ & C+ AGT™ dry media format Nutrient Supplement
- FunctionMAX™ TiterEnhancer Additive

Thermo Fisher Scientific (Gibco®) can also custom manufacture and/or otherwise optimize these and other AGT™ dry media format culture media for clients on a custom basis. This includes Gibco® PD-Direct® Services (process design); Gibco® Media Express™ (rapid, 10-15 days, manufacture of small quantities); and Gibco® cGMP Media Custom Services (custom media for commercial manufacturing).

AGT™ dry media format culture media are complete, or as complete as a single dry high-performance mammalian culture media formulation can be, with at most one or a few supplements needed. This includes standard supplementation with L-glutamine for cell lines/expression systems requiring this and supplementation with cholesterol for NS0 cell culture. Besides essential nutrients, AGT™ dry media format may already contain recombinant insulin, other growth factors and supplements typically added separately as supplements to DPM. Also, AGT™ dry media format upon mixing requires no adjustment of pH and osmolality, with this simplifying its preparation. And besides being fully chemically-defined, AGT™ dry media format products are serum and other protein-free; fully animal origin-free (AOF); and contain no hydrolysates or other complex, variable composition, components. [Note: one AGT™ dry media format formulation, VP-SFM / OptiPro AGT™ dry media format, designed primarily for VERO cells, does include non-animal hydrolysate supplementation].

AGT™ dry media format products use and performance in cell culture are comparable to other competing products, including to the ‘same’ combination of ingredients in other media formats – DPM and manufacturer-prepared bulk liquids. Yields and titers are generally equivalent or better than competing products and formats. AGT™ dry media format media are consistent and readily scalable, with the same media able to be used

from micro-well through world-class commercial manufacturing scales, with multiple marketed products manufactured using AGT™ dry media format media. AGT™ dry media format media show lot-to-lot consistency, at multiple lot/batch manufacturing scales. AGT™ dry media format media work well with most mammalian hosts cells, including common research and production mammalian host cell lines, e.g., CHO, HEK293, hybridomas, NS0 and VERO cells.

The proven equivalence of AGT™ dry media format dry and liquid media facilitates adoption of the dry format, even at the smallest scales. For example, an AGT™ dry media format formulation, whether stock/catalog or custom manufactured, can be packaged as hydrated liquid, e.g., in 1 L bottles, for early R&D studies, screening, etc., with the user seamlessly converting to standard dry AGT™ dry media format as bioprocessing is scaled-up. AGT™ dry media format media have been documented and user experience confirms that most any suitable-scale mixer, whether single-use or stainless steel, can be used for AGT™ dry media format liquids preparation. AGT™ dry media format granules are so readily soluble that mixing protocols have essentially no effect, result in no inconsistencies, in prepared liquid media.

All AGT™ dry media format manufacture is performed in cGMP facilities that are maintained animal origin-free (AOF). AGT™ dry media format media manufacture, including custom formulations, can be manufactured in lots ranging from 50 to 6,000 kg. AGT™ dry media format media are manufactured at facilities in Grand Island, NY, and will in the future also be produced in Inchinnan, Scotland. Between redundant manufacturing facilities and AGT™ dry media format being the leading mammalian cell culture product line from a leading culture media company, users have come to expect a reliable supply chain. A variety of packaging options are available, all single-use, including bags,

drums and kegs up to 150 kg. Options include tapered bottle-shaped bags for easy transfer, tri-clover connectors; anti-static film to further improve AGT™ dry media format granules flow and transfer; and custom pre-weighed bags for simple attachment and addition to mixers.

Full regulatory documentation is available for AGT™ dry media format products, including DMFs in place with the FDA. Regulatory agencies worldwide are familiar with and have approved products manufactured using AGT™ dry media format media, from INDs through product approvals.

End-user Problems with DPM

In researching this White Paper, industry end-users identified problems and issues in the use of conventional DPM. These dry powdered media, composed of heterogenous, non-fused, finely ground, individual nutrient particles, continue to dominate the industry. However, problems inherent with DPM continue to create challenges.

DPM-associated problems can be alleviated through use of AGT™ dry media format, which increases productivity, saves time, decreases bioprocessing and regulatory risks, simplifies supply chains, provides increased flexibility and simplicity, and reduces costs. AGT™ dry media format also provides increased flexibility and speed-to-market, increasingly the top priorities for biopharmaceutical development.

Problems that AGT™ dry media format addresses include:

- ***Inconsistencies in manufacture*** – Users report the ‘same’ DPM manufactured at different scales, using different equipment, are not fully consistent, with this often resulting in variations in cell culture.
- ***Increased costs*** – AGT™ dry media format media are available as stock/catalog items. As such, these tend to be less expensive compared with customized culture media; custom manufacture can significantly increase the price [2].
- ***More nutrients needed*** – Conventional DPM typically do not include all nutrients, growth factors and other supplements, incorporated into AGT™ dry media format granules. Typically, 8-15 additional nutrients must be added to the basal media during mixing of powders and/or as supplemental feeds during bioprocessing. This can be avoided with AGT™ dry media format, and users have the option of further supplementing their media.
- ***More time and money required*** – Conventional DPM is reported by users (see below) as requiring about twice as much staff and time in its preparation compared with AGT™ dry media

format. The format also does not form dusts, clumping and is more water soluble. So facilities have found they can save considerable staff time and related costs.

- ***Less suitable for platform adoption*** – With the added flexibility AGT™ dry media format provides compared to DPM, particularly ease of preparation, and high-performance as a complete culture media, AGT™ dry media format can be adopted as facility- or company-wide platform culture media, allowing savings from purchase of larger volumes of AGT™ dry media format and use of fewer media.
- ***pH and osmolality need to be adjusted*** – DPM (and also liquid media concentrates) preparation generally requires addition of acid(s), base(s) and/or salt(s) to adjust it to the desired pH and osmolality. Agents used to adjust these properties, e.g., HCl and NaOH, present significant hazards to process operators. AGT™ dry media format is pre-formulated to be at optimal pH and osmolality upon hydration.
- ***Inconsistencies in liquid preparation*** – Problems due to variations in preparation of DPM can be avoided, with AGT™ dry media format more water soluble than DPM.
- ***Difficulties in mixing*** – DPM presents problems in mixing related to its lower solubility vs. AGT™ dry media format, including clumping, powders floating on the surface, etc.
- ***Longer turnaround times*** – DPM preparation is reported by users (see below) to require on the order of twice as many hours (and staff) as comparable volume AGT™ dry media format preparation. Using the format can mean same-day bioprocessing, rather than media preparation on day-one, followed by refrigerated storage. AGT™ dry media format use vs. DPM has been reported to double in-house culture media preparation productivity; a considerable cost savings.
- ***Different media formulations used at different scales*** – DPM often involves manufacture and formulations that are different at different scales, including differences as media use progresses to larger scale manufacture. In contrast, AGT™ dry media format manufacture provides comparable media at all manufacturing and preparation scales, enable it to be adopted from initial discovery, bioprocess design, through clinical and commercial manufacture.
- ***More quality control tasks are required*** – Each of the often 8-15 supplements typically added to basal DPM requires its own raw materials testing and clearance. Use of AGT™ dry media format, as a single complete media, with generally none or at most a just a few supplements added, can substantially reduce media related quality control tasks.
- ***More problems with optimization*** – AGT™ dry media format is a complete media that provides high performance even without supplementation, so optimization may not be needed. With liquid media from DPM being both incomplete and less consistent in manufacture and preparation, AGT™ dry media format offers higher yields through media supplementation and optimization.
- ***Safety/toxicity hazards*** – DPM powders typically form dusts when handled and transferred, requiring operators to wear protective gear to prevent inhalation and ocular toxicity. AGT™ dry media format, composed of larger multi-component powder agglomerated granules, avoids these problems.
- ***DPM is 'messy'*** – DPM preparation tends to make more of a mess, requires more cleanup, than AGT™ dry media format preparation. DPM powder transfer is more prone to spilling and spreading, compared to larger granule AGT™ dry media format. Also, with fewer additions and

- faster mixing, spillage of powders or liquids is decreased and cleanup is simpler.
- ***Sterile filtration can be problematic*** – Liquid media is essentially always subjected to sterile filtration prior to input into bioreactors. Conventional DPM, including when not fully and properly hydrated, can result in retention of particulates, clogged of filters and other problems. With its higher solubility, use of AGT™ dry media format enables easier sterile filtration.
 - ***Increased supply chain complexity and related costs*** – Use of DPM generally requires use of diverse supplements, often from different sources. This complicates supply chain management and increases costs, including requiring qualification and audits of more manufacturers/vendors.
 - ***Supply chain reliability problems*** – Market research shows that most DPM used in biopharmaceutical development is custom manufactured. Generic DPM, e.g., DPM versions of products commonly sold by diverse vendors in liter bottles, are rarely used in bioprocessing beyond initial process design. In contrast, AGT™ dry media format is an established popular product line, the lead mammalian cell culture products from the culture media industry leader, both in terms of sales and innovation. With AGT™ dry media format products, particularly, stock/catalog products, being high volume and coming from the culture media market leader with redundant manufacturing facilities (discussed below), AGT™ dry media format offers better supply chain reliability.
 - ***Documentation is more complex*** –DPM use involves more media components and additions. This requires more regulatory documentation, besides the additional quality control testing of each component cited above.
 - ***Inventory management is more complex*** – Using much fewer media components, as enabled by AGT™ dry media format, simplifies inventory management and reduces related costs.
 - ***Complex natural product ingredients add to media inconsistency*** – Any animal- or plant derived complex mixtures, such as hydrolysates, make resulting media for inconsistent among batches/lots, both DPM prepared by manufacturers and liquids prepared by end-users. AGT™ dry media format does not contain these ingredients, performs well without them, and avoids these problems.
 - ***Lot-to-lot inconsistency*** – DPM products generally are prone to more lot-to-lot inconsistencies than AGT™ dry media format products. Lot-to-lot consistency of manufacture is well-established, with the product on the market for a decade and many users confirming this benefit.
 - ***Problems in scale-up*** – Scalability is more of an issue with DPM than with AGT™ dry media format, with both preparation and use being simpler and more reliable. AGT™ dry media format tends to be more scalable, with its higher solubility and consistency of both manufacture and in-house preparation of liquids, including at different scales and with both stock/catalog and custom products. AGT™ dry media format also does not require multiple supplements, which can complicate scale-up.
 - ***Less regulatory-friendly*** – DPM, with its variability in manufacture and end-user preparation, can be more problematic to regulatory agencies. Regulatory agencies worldwide are familiar with AGT™ dry media format, and it is commonly used with IND/clinical stage products and used for manufacture of multiple marketed products. In fact, among culture media products from Thermo Fisher Scientific (Gibco®), the market leader in culture media, “AGT™ dry media format comes with the highest level of testing for all cell culture media.”

- *More training required* –When compared with DPM, AGT™ dry media format preparation and use tends to be simpler, so related staff training time can be substantially reduced.
- *More equipment maintenance* – DPM involves much longer mixing than AGT™ dry media format, with less wear and more batches (higher productivity) before equipment needs to be taken off-line for maintenance.
- *More media batches/lots* –AGT™ dry media format is broadly adaptable for platform use, so fewer culture media preparation runs may be required. The same batches/lots of prepared AGT™ dry media format liquid media can be used for multiple bioprocesses, including multiple bioreactors producing the same or different products and/or at different scales.

AGT™ Dry Media Format as an Alternative to DPM

The alternatives to use of conventional ground powdered media (DPM) that is then rehydrated to liquid media, by end-users include:

- 1) Supplier-prepared bulk liquid culture media, and
- 2) Alternative powders, such as AGT™ dry media format.

Bulk liquids are an increasingly attractive option for many users, particularly at pre-commercial scales [2]. However, as noted above bulk liquid media cost significantly more than comparable powders, with the user having to pay for shipping of what is mostly water (WFI) and also pay for the media company to manufacture a finished product, including the preparation and quality control tasks. Few liquid media are stock/catalog items at larger scale, with most all liquid media custom manufactured, and generally requiring weeks for delivery. In contrast, powders (both DPM and AGT™ dry media format) can be prepared in-house for immediate use. And because bulk liquids have a much shorter post-hydration shelf-life, users lose the inventory flexibility powders provide through their longer shelf-life. AGT™ dry media format, for example, can be stored and used for two years. AGT™ dry media format offers many the benefits of liquid media – flexibility, convenience, consistency, etc. – but without the added liquid storage, transportation other issues and costs. AGT™ dry media format culture media, compared to conventional DPM and bulk liquid media, can substantially reduce many of the costs and problems with these other media formats, while also providing more consistent culture media and simplifying preparation tasks.

AGT™ dry media format can be readily adopted as a ‘platform’ or standardized media to be used with multiple products at diverse scales, either using stock AGT™ dry media format, customized AGT™ dry media format formulations, or using AGT™ dry media format (either stock or custom) as the basal medium to which one or more supplements are added. End-user experience confirms this (see below). Traditionally, the general approach to culture media selection involves customization/optimization of DPM, with each product’s/processes’ DPM individually customized for its bioprocessing, with this adding considerable to costs. While the classic individual process approach to media optimization enables optimization of performance and cost benefits, industry increasingly prefers flexibility, e.g., as exemplified by single-use vs. stainless steel bioprocessing equipment, and standardization across facilities and products/processes.

AGT™ dry media format has been designed for simplicity and consistency, even when prepared by diverse facilities using different equipment, and can be adopted as a ‘platform’, preferred facility mammalian cell culture medium. And of course, if desired, AGT™ dry media format can be further supplemented, including with proprietary growth factors and other supplemental nutrient feeds and can be custom manufactured, including process-specific optimized culture media.

More than 90% of large-scale culture media is purchased from manufacturers as ground powders, primarily conventional DPM, for in-house mixing. Nearly all bioprocessing facilities are currently designed to handle powders. About 20% of space in facilities operating at larger scales is typically devoted to culture media (powder storage, mixing, liquid storage, etc.). Larger facilities, particularly those operating at commercial scale, generally have bioprocess operators spending all or much of their time preparing liquid media from DPM.

DPM-using facilities often view in-house media preparation from powders a standard process that can even-out schedules. Thus, adoption of AGT™ dry media format, like DPM a powder requiring weighing, transfers, hydration/mixing, sterile filtration and other media preparation tasks, provides much the same benefits in terms using preexisting DPM-related facilities’ infrastructure.

AGT™ dry media format culture media, compared to conventional DPM, can substantially reduce many of DPM's downsides and additional costs, while simplifying preparation tasks. AGT™ dry media format can be readily adopted as a 'platform' or standardized media to be used with multiple products, either using stock, customized formulations, or using AGT™ dry media format (either stock or custom) as the basal medium to which one or more supplements are added. Traditionally, the pattern of culture media use involves customization/optimization of DPM media on a product-by-product (process specific) basis, with each product's culture media individually customized for its bioprocessing. While this classic one-off approach to media optimization allows optimization of performance and cost benefits, industry increasingly prefers flexibility, e.g., as exemplified by single-use vs. stainless steel bioprocessing equipment, and adoption of platform technologies. AGT™ dry media format has been designed for simplicity and consistency, even when prepared by diverse facilities using different equipment, whether stainless steel or single-use, and can be readily adopted as a 'platform' mammalian cell culture media.

Launched a decade ago, AGT™ dry media format has become a popular culture media option, and remains the fastest-growing segment among Thermo Fisher Scientific's (Gibco®) cell culture media products, with a secondary site coming online soon.

Case Study 1: Overall Benefits from Cost Reduction

Reductions in costs in process development through scale-up

- o Customers using AGT™ dry media format spend less time and use fewer resources
- o Reduction and prevention of formulation errors due to single component media (vs. multiple components & add-backs)

An example of a facility adopting AGT™ dry media format media for its overall benefits, including cost reduction, is Morphotek, Inc. (Exton, PA), now a subsidiary of Eisai Co. Ltd. Morphotek is developing innovative fully-human recombinant monoclonal antibody (mAb) products using MORPHODOMA and other proprietary mAb engineering technologies. The company currently has five mAbs in clinical trials. Facilities include a 60,000 sq. ft., \$80 million, pilot plant that came online in late 2012, used for bioprocess development and manufacture of clinical supplies.

The facility was designed with state-of-the-art highly automated, scalable bioreactors, using both fixed and disposable technologies, with associated downstream purification capability, that enable the rapid scale-up and optimization of processes from lab to 1,000L bioreactor scale. The facility supports multiple products concurrently, with multiple suites. Besides supporting the company's own preclinical and clinical programs, Morphotek seeks strategic partners needing GMP manufacturing services.

Mr. Frank Lee, Director of Upstream Operations, Clinical Pilot Plant, at Morphotek reports having used AGT™ dry media format products since 1999. This decision to adopt an AGT™ dry media format platform occurred even before the company's product line had its formal launch in 2004. Morphotek adopted AGT™ dry media

format after comparing it to other dry media (DPM). Mr. Lee noted that the format offered specific, compelling advantages for his operations, all of which contributed to lower operating costs, including:

- *Time/money-saving* – Morphotek reports at least 50% reduction in labor time and costs. Compared to DPM, AGT™ dry media format preparation involves half the amount of staff, e.g., 2 vs. 4 FTE (operators), and half the time, e.g., a half day vs. full day, to comparably complete all mixing-related tasks. Initial AGT™ dry media format dissolution is reported to be 2x at fast as DPM.
- *Requiring less space* – As a smaller (pre-commercial manufacture) facility and desiring to avoid needed additional space and infrastructure, e.g., heavier-duty mixers, for preparation of liquid media from DPM, the only other options were manufacturer-prepared bulk liquid media, with downsides including the requirement for considerable refrigerated storage space and higher purchase costs; liquid concentrates, which are rarely used, incomplete, and present their own mixing problems; and AGT™ dry media format.
- *Operational safety* – Culture media powder and prepared liquid movement, handling, weighing, mixing, pumping, etc. are significantly less hazardous/safer with AGT™ dry media format, compared to DPM. The facility determined that AGT™ dry media format use would result in less mess and spillage; any spillage would be easier to clean up; require less or no environmental air monitoring, e.g., dusts; and operators would have less need for restrictive goggles, masks, etc.
- *Stock/off-the-shelf* – Morphotek uses stock/catalog AGT™ dry media format for its multiple products at all scales. This is both less expensive than custom media and provides the convenience and reliability of wide availability using in-stock, high-volume products.
- *Consistency* – Mr. Lee reports that AGT™ dry media format provides consistently good performance, including at different scales and with different products. Having adopted AGT™ dry media format and finding it satisfactory, no extensive comparative testing vs. other culture media has since been performed.
- *Longer storage (shelf-life)* – Mr. Lee confirmed that AGT™ dry media format

has a longer useful lifespan than most other powder media.

- *Fewer supplements/feeds* – Mr. Lee confirmed use of much fewer supplements/feeds with AGT™ dry media format – just a few, vs. many, e.g., often 8-12 with DPM. Mr. Lee also confirmed that AGT™ dry media format provides competitive high yields with consistent, predictable bioprocessing and products.

Ultimately, the decision to adopt AGT™ dry media format media was based on overall operating cost reductions and ease of use in process development through scale-up. Morphotek, Inc's recombinant monoclonal antibody (mAb) products required the ability to be rapidly scaled-up and optimized from lab scale to 1,000L bioreactor scale. Morphotek's use of AGT™ dry media format products was based on the specific advantages for his operations, and the overall lower operating costs.

Case Study 2: Process Comparison Leading to Conversion

Comparison of AGT™ dry media format vs. other dry media

- Benefits of specific features, including ease of use, auto adjusting pH & osmolality, single part medium, fast hydration time, etc.
- Benefits of general attributes including batch-to-batch lot consistency

AGT™ dry media format performance in cell culture has been shown by end-user evaluations to be comparable, if not better, than most other similar competing products. This has been confirmed by many users, with the format a popular media option that continues its rapid market growth.

Dr. Hao Chen, Principal Scientist, BioProcess Development, Merck & Co., Inc. (Rahway, NJ) reports his facility adopted AGT™ dry media format as a platform mAb media after comparative testing against other products. In doing head-to-head comparisons with other media prior to adoption, Merck found typical 20%-30% improvements with AGT™ dry media format in cell culture performance. For example, improved titers with no change in product quality or characteristics; such changes are a common experience in the use of other DPMs. In comparing AGT™ dry media format against Merck's own proprietary and other commercial mAb media, AGT™ dry media format often came out at the top. The format is currently used at the Rahway facility at up to 200-500 L scale for clinical-scale mAbs production. No products using AGT™ dry media format have yet advanced to commercial production, but this is expected to change. With AGT™ dry media format, feeding is done once-daily vs. with other media products must be done 2–3x daily. A single feed does what multiple feeds of other media do. Also, feeds are lower in volume (more concentrated), providing easier operations.

For Dr. Chen and his facility, AGT™ dry media format is now the in-house reference standard against which other media are compared. The single most important problem Dr. Chen cited as having been resolved using this format was cell culture performance. AGT™ dry media format use provided overall better performance compared with other media. Cell culture performance was the primary concern of his department at the time. He confirmed that AGT™ dry media format preparation/mixing requires less labor and is easier vs. DPM. His facility continues to look for better media, and AGT™ dry media format remains overall the best for their mAbs manufacture.

Other facilities have experienced similar results. Ms. Toni Hill, Director, Product Development, Fujifilm Diosynth (Research Triangle Park, NC), also reports having selected AGT™ dry media format after comparisons with other media. This facility manufactures mAbs for Fujifilm's own cancer immunodiagnostics and also sells mAbs to other diagnostics manufacturers. However, unlike most mAbs, which now use transformed/recombinant cell lines, bioprocessing involves classic cell culture production of non-recombinant, fused-cell, hybridomas expressed mAb. Most of these mAbs were originally produced using the classic ascites culture method - culture of hybridoma cells in the peritoneal cavity (belly) of mice. But related problems included the hybridomas often inducing tumors in mice, reducing productivity, requiring extra work/inspections and documentation. The facility replaced this with hybridoma cell culture in stirred tank bioreactors.

FujiFilm's bioprocessing using AGT™ dry media format currently involves hybridoma cell culture in 15 L stirred-tank bioreactors with perfusion (Applikon Biosep). Cost savings from the switch in antibody production methods were

estimated at over 30%, and productivity increased noticeably as well. Going animal-free in hybridoma culture allowed the facility considerable savings from simplified, fewer steps, purification to remove mouse proteins. The facility selected AGT™ dry media format after testing many media, with the format providing the highest yields, cell densities, good antibodies, etc. among all testing, plus their cell culture is animal-free. No feeds or supplements are used. The single most important problem resolved by AGT™ dry media format cited by one respondent, was the ability to eliminate “junk” animal proteins – and achieve animal-free antibody manufacture. The facility produces many types of antibodies for different kits and resale to other kit manufacturers. AGT™ dry media format has been found to work well with all the diverse hybridomas used at the facility.

Case Study 3: CMO Perspectives

CMOs perspective: Why CMOs would choose AGT™ dry media format?

- o Product's reliability/robust process with repeatable results
- o Ease of use

Why Would CMOs Choose AGT™ dry media format?

AGT™ dry media format is ideally suited for Contract Manufacturing Organization (CMO) adoption as a preferred platform mammalian cell culture media. The flexibility, time and cost savings offered by AGT™ dry media format are very attractive, and in some cases essential, to CMOs. This is because contract manufacturers are concerned with both high quality, and cost-effective bioprocessing for their clients. In addition, they need to lower costs and increase productivity to be competitive against other CMOs. All of which are looking for ways to reduce their costs and improve productivity. CMOs have found they can adopt AGT™ dry media format for a range of new mammalian cell culture projects that are presented by prospective clients. Wherever clients allow, they can expect that AGT™ dry media format will satisfy most client project needs, including using stock/catalog, non-custom manufactured AGT™ dry media format media.

Was AGT™ dry media format adopted for the product's reliability/robust process with repeatable results?

CMO AGT™ dry media format users interviewed responded strongly with a "Yes!" CMOs appear to expect that AGT™ dry media format will work as well, if not better

than any other single culture medium, will perform well, comparable to supplemented DPM, right from the start. This appears to be the case whether AGT™ dry media format is adopted at discovery, bioprocess design, clinical or commercial manufacturing stages. Being able to adopt a single preferred platform medium across many projects and over many years, with multiple diverse client projects is important, especially to CMOs. It enables further in-house fine-tuning of AGT™ dry media format preparation and bioprocessing, which can be an invaluable benefit for CMOs and their clients. And similarly, where CMO clients are already using AGT™ dry media format, they can trust that CMOs will have no problems adopting use of AGT™ dry media format, including assurance that their CMO will prepare liquid media that is comparable (in the strict regulatory sense) with that the client uses in-house.

Ease of use?

CMO AGT™ dry media format users similarly responded with a strong “Yes!” Ease of use includes AGT™ dry media format preparation being quicker and simpler, compared to conventional DPM. About half the staff are needed for preparation, and total preparation time is about half of what is needed for DPM. The rapidity of AGT™ dry media format dissolution and the lack of powder clumping, dusts and other problems makes AGT™ dry media format preferred in the busy CMO production environment. With its relative ease of both preparation and use, AGT™ dry media format is competitive with or as close as a powder media can get to use of bulk manufacturer prepared liquid media, but which costs much more. Overall, a number of CMOs have noted that AGT™ dry media format can be the single most cost-effective culture media for a contract environment.

CMO Case Studies

Mr. Phil Garcia, previously with Laureate Pharma (Princeton, NJ), a mammalian cell culture-based CMO recently acquired by Gallus Biopharmaceuticals, confirmed positive experience at Laureate with AGT™ dry media format. This included his noting that preparation of liquid media takes about half the time with AGT™ dry media format vs. DPM. Preparation of 2,000L from competing DPM powders typically takes a full day with four staff involved, while AGT™ dry media format can be prepared in half the time using the same four staff.

The upstream manager at a large mid-West U.S. CMO noted that the primary advantage AGT™ dry media format provides for his company is storage and, related to this, availability. The CMO uses stock/catalog AGT™ dry media format (no custom orders), due to the fact that it is critical that there be no waiting for custom media to arrive (which can sometimes require weeks of production planning). The facility has limited storage space, so rapid, as-needed delivery is a definite benefit. The upstream manager also reported an estimated 25% reduction in costs and time for media preparation. Solubility was identified as the primary benefit, with shorter mixing time. The upstream manager also confirmed his experience as a CMOs operator with multiple projects in which AGT™ dry media format reduced media supplemental feeds to one (or two) from usual 8-10 components required with DPM. This reduction in feeds means simpler operations, involving less contamination risks, less work, and a media end result that is more consistent. The CMO regularly uses stock AGT™ dry media format media along with other media for its clients' projects, including use at 50-1,000L scales in both single-use and stainless steel bioprocessing systems, including for Phase I and II trials. The upstream manager recommends AGT™ dry media format as fully suitable for adoption by CMOs.

Conclusions

Numerous decision factors affect the decisions regarding selection of dry powder media (DPM) vs. AGT™ dry media format. The three case studies from multiple end-users provide experiences related to improved performance by using AGT™ dry media format, and the key factors that influenced decision-making.

As discussed in this White Paper, AGT™ dry media format culture media offers significant benefits compared to other media formats, including conventional DPM; manufacturer-prepared bulk liquid media; and liquid media concentrates. These include:

- AGT™ dry media format media preparation and use are simpler, less expensive, faster and more consistent.
- This can make AGT™ dry media format an attractive in-house platform for mammalian cell culture media applications.
- AGT™ dry media format media have been, and continue to be, the fastest-growing media product line from Thermo Fisher Scientific (Gibco®)
- Users recognize AGT™ dry media format media quality and its supplier's robust supply chain.

For further information, contact your Thermo Fisher Scientific media sales representative and/or visit www.lifetechnologies.com/agt

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