Development & Technology Transfer of a cGMP Potency Assay: Testing of an Ancillary Material for Stem Cell Manufacturing

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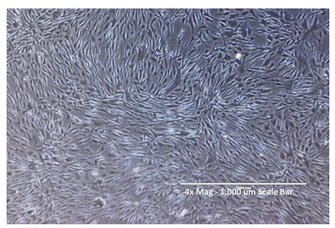
BACKGROUND

- The over 800 active clinical trials involving mesenchymal stem cells (MSCs) indicate an upcoming wave of demand for clinical-grade cells and medium.
- Products manufactured for clinical use require cGMP manufacturing and rigorous analytical testing to ensure product robustness, reproducibility, and patient safety.
- We successfully established a custom cGMP MSC-based potency assay.
- The initial development was in-house and then transferred for regular cGMP testing at a Contract Testing Organization (CTO).
- For our CliniControl™ medium supplement, this potency assay is based on cell growth, evaluated as calculated fold expansion of the final cell population number over the initial cell number.
- This potency assay currently serves as one of the product specifications (i.e. release criterion), as well as part of the stability studies in support of shelf-life determination.
- Ongoing maintenance and continuous improvement will be important in preserving this cGMP assay.

CELL EXPANSION ASSAY

Test article:
RoosterBooster™-MSC-CC

Reagents:
RoosterBasal™-MSC-CC
RoosterVial™-hBM-20M-CC



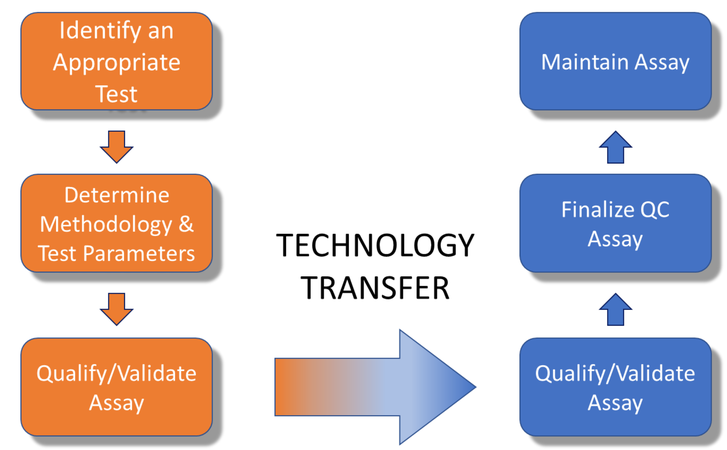
- A potency assay of the medium supplement was needed to serve as a release assay for this ancillary material that supports cGMP stem cell manufacturing.
- The test article is a frozen supplement configured to be added to a basal medium, which together serve as a complete medium to support MSC growth.
- Performance of the medium supplement is inherently linked to both the basal medium and cells, reagents that add complexity in assay design.
- A number of assays were considered and ultimately a proliferation-based assay was chosen.

GENERAL ASSAY PARAMETERS

Parameter	Options
Cell Type	Immortalized Cell Line, **Primary Cells**
Culture Duration	**Defined Period**, Confluency-Based, Biomarker-Based
Assessment	Replicative DNA, Metabolic Activity, **End-point Cell Count**

- Proliferation-based assays have a number of parameters to be considered.
- Cell selection includes the choice of immortalized vs primary cells. While immortalized cell lines may be more stable, we elected to use primary MSCs as the most relevant cell type. Additional considerations then included passage level, as well as seeding method (e.g. cell density and culture vessel).
- The culture duration may be a prescribed defined period of time or a confluency-based decision point by a trained operator. While confluency-based culture allows for more flexibility, culture duration for a fixed period of time (e.g. 4 days) was selected due to assay reproducibility.
- Proliferation can be evaluated using a wide variety of assessments, such as S-phase determination or BrdU for replicative DNA, mitochondrial activity for metabolic functionality, or increase in cell number for whole population expansion. Here we selected an assessment based on cell counting, which has been used in numerous cGMP assays.
- The quantitative metric used as the product specification was then chosen to be mean fold expansion, a calculated value of final cell number divided by the initial number of cells seeded.

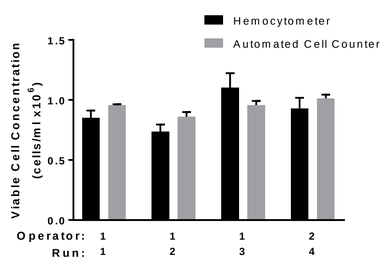
ESTABLISHMENT OF A cGMP ASSAY



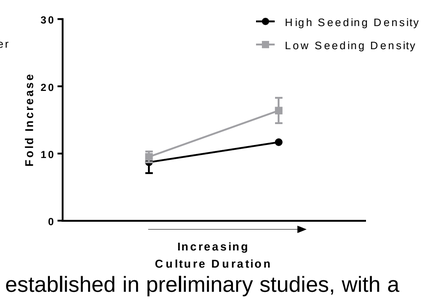
- Without in-house cGMP facilities, it becomes necessary to undergo a technology transfer to a contract testing organization (CTO) for all cGMP assessments.
- In development, it is important to identify a test that properly addresses the testing need.
- Preliminary studies establish assay methodology and test parameters.
- To qualify or validate the assay, a list of assay properties should be evaluated, including Accuracy, Precision (Repeatability and Intermediate Precision), Specificity, Robustness, Linearity, and Range.
- Once the assay has been established in-house, it is transferred to a CTO.
- The CTO will repeat qualification or validation, based on the rigor requested, at their own facility.
- Finalizing the QC assay consists not only of establishing technical details, but also documentation including protocols and master batch records.
- Over time, the assay will require active maintenance, including occasional requalification or revalidation, due to changes in reagents or reference standards.

ASSAY METHODOLOGY AND TEST PARAMETERS

Counting Method

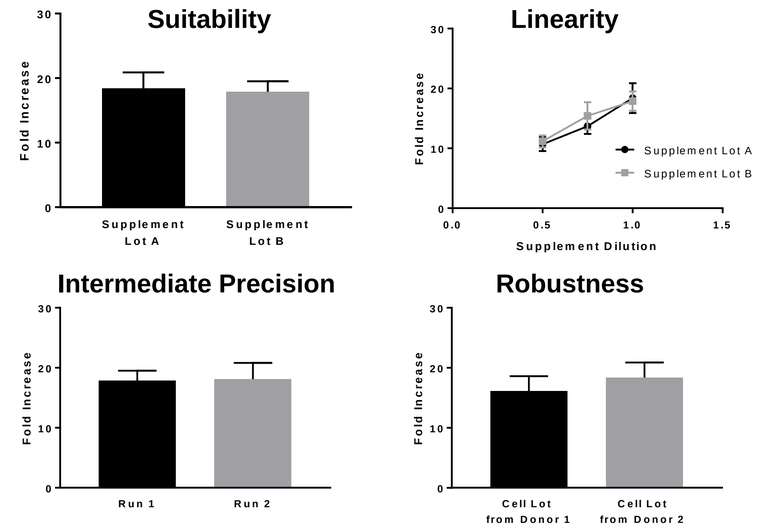


Culture Parameter



- Specific assay and test properties were established in preliminary studies, with a selected number shown here.
- As proper determination of cell number was crucial for this design of the assay, multiple methods were evaluated.
- The hemocytometer had been used in initial studies, so a direct comparison was necessary of this established but operator-dependent method to the adapted use of the NucleoCounter NC-200, a semi-automated device that is GMP and 21 CFR Part 11 compatible.
- Empirical data was used to select cell seeding density and culture duration.

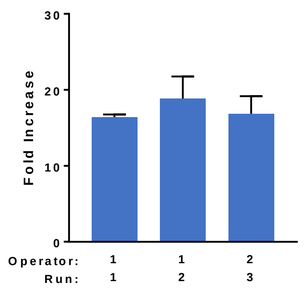
ASSAY DEVELOPMENT



- Assay development requires a wide range of studies, with just a limited number of examples discussed here.
- Suitability testing evaluated whether the assay could perform as expected across different supplement lots.
- Linearity of the assay was evaluated by performing the assay with different dilutions of the medium supplement, repeated with 2 lots of production.
- Intermediate precision was evaluated by having the assay performed across different operators and runs over a span of time.
- Robustness testing included small deliberate changes such as altering the cell lot, a biological reagent in this assay configuration.

ASSAY TRANSFER

- A major effort was required to ascertain the proper transfer of technology to the CTO, necessitating iterative review of drafts of protocols and batch records.
- As a qualified assay was to be established, the CTO repeated some of the testing previously executed during development.
- Intermediate precision, including three runs and two operators was performed.
- Ultimately, the CTO will finalize test parameters and documentation prior to testing.



MAINTENANCE OF ASSAY

- In addition to release testing, the potency assay is also being used in stability studies, with samples from the same lot tested after various storage periods.
- Determining the duration over which samples from the same lot maintain critical quality attributes aids in establishing the shelf-life of the product.
- Proper maintenance of the assay is needed over months-to-years to allow release of numerous production lots or direct comparison of samples in stability studies.
- One particular challenge in maintaining this assay is it includes basal medium with a shelf-life of only 1 year, thus requiring annual assay requalification.

CONCLUSIONS

- A cGMP Potency Assay was successfully developed and transferred to a contract testing organization.
- The established assay is currently used as a product specification for an Ancillary Material to Support cGMP Stem Cell Manufacturing.
- A Master File has been submitted to the FDA in support of this medium supplement.

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