RESULTS OF GMP COMPLIANT KARYOTYPE STUDIES TO SUPPORT CELL THERAPIES

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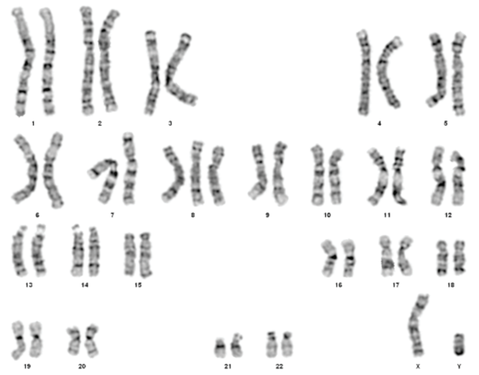
INTRODUCTION

G-banded karyotyping is a cytogenetic technique used to assess chromosomal number and structure at the single-cell level (see Figure 1). Karyotype has long been accepted as appropriate for assessing the genomic integrity of pluripotent stem cells and their derivatives for both research and translational work. It is included in the ISSCR Standards for Human Pluripotent Stem Cell Research as an appropriate test for assaying the genome (Ludwig et al., 2023) and is used for release testing for clinical cell banks, including hPSC derived and other cell-based drug products.

WiCell has been performing cytogenetic analysis for the scientific community since 2006 and has performed more than 40,000 karyotype assays to date. In the last 5 years alone, over 20,000 karyotype analyses have been performed, demonstrating the significant uptake of the assay as a method of monitoring cells through the research and development. With an increasing number of researchers entering the translational pipeline (Kirkeby et al., 2025), WiCell established a GMP compliant assay for g-banded karyotype to better support clients considering advancing therapies and regulatory filings.

FIGURE 1: Example Human Karyogram with Trisomy 8



WiCell presents data here on the uptake of this GMP service for stem cell specific cell therapy products. Additionally, the abnormality rates reported for standard (non-GMP) services with the GMP-compliant services performed in support of cellular therapy applications are noted and discussed.

STUDY DESIGN

This is a retrospective analysis of work performed over the last 5 years in the Cytogenic Laboratory at WiCell. It examines the total number of GMP-compliant karyotypes performed annually and compares the abnormality rate of materials submitted for cGMP compliant analysis vs. those submitted for standard services. Because these data were not developed experimentally, but rather through regular services, there is limited ability to make conclusions about the cause for abnormalities noted. The authors acknowledge ascertainment bias in the samples represented here. For example, some samples submitted for non-cGMP karyotype testing may be from known abnormal cell lines or may contain expected karyotypic changes. This likely represents a small proportion of the results included in this retrospective study.

MATERIALS AND METHODS

Protocols for karyotype analysis have been previously published (McIntire et al. 2021). Briefly, actively dividing cells are cultured and arrested in metaphase, when chromosomes are condensed. Cells are then detached from culture vessels, treated with hypotonic solution, and then fixed using Carnoy's fixative. After fixation, the cells are dropped onto microscope slides and treated with trypsin and stained with Leishman's stain, producing a characteristic pattern of light and dark bands unique to each chromosome. Standard g-banded karyotype analysis at WiCell includes 20 metaphase cells, however some analyses contain more cells based on specific project requirements. These banding patterns, analyzed under a microscope by clinically trained cytogenetic technologists, allow for the detection of structural abnormalities (such as translocations, deletions, duplications, and inversions) as well as numerical changes (aneuploidy, polyploidy). Results are reported according to the International System for Human Cytogenomic Nomenclature (ISCN).

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WICELL SERVICE OPTIONS

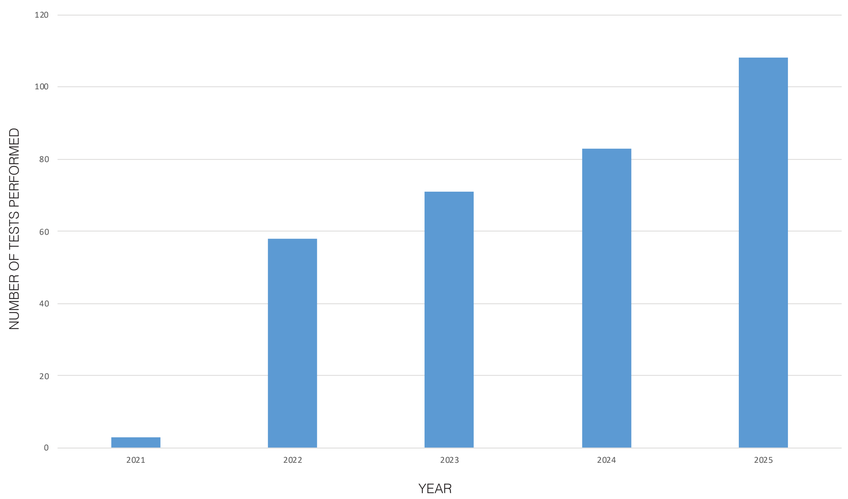
ATTRIBUTES	NON-CGMP SERVICE	CGMP SERVICE
Intended Use	Recommended for Research Product Characterization	Recommended for Translational and Clinical Product Characterization
	Use for research and development and to familiarize your team with the service and results.	Used to verify the quality of cellular materials for inclusion in regulatory submissions.
Compliant with 21 CFR 211	No	Yes
	WiCell holds quality as our top priority. Our quality system consists of a dedicated Quality Assurance Team ensuring compliance, supplier management, and a robust training program.	
Personalized Customer Technical Support	Yes	Yes
Supplier Qualification	Yes	Yes
Harvest Reagent Traceability	Yes	Yes
Harvest Batch Record	No	Yes
Quality Questionnaire *(if requested)*	Yes	Yes
In-Person Audit *(if requested)*	No	Yes
Quality Agreement *(if requested)*	No	Yes
Prospective Process Description	No	Yes
	A process description allows for work to be customized by cell type, project, and is pre-approved by the client.	
Method Qualification Available	No	Yes
American Society of Clinical Pathology (ASCP) Certified Analyst	No*	Yes
	*All WiCell karyotypes are analyzed by two analysts (at least one of whom is ASCP certified) and QC reviewed by a third analyst.	
Deviations Reported	No	Yes
Publication Images *(if requested)*	Yes	Yes
Final Test Report	Yes	Yes
	All final karyotypes are reviewed and signed by an American Board of Medical Genetics and Genomics (ABMGG) board certified or board eligible director.	
Final Test Report with QA Review	No	Yes
Certificate of Compliance	No	Yes
Data Package Available	No	Yes
Custom Report Writing Support Available	No	Yes

RESULTS AND DISCUSSION

The use of cGMP assays in support of clinical trials and regulatory filings has increased significantly over the last 5 years (Figure 3). The additional oversight and documentation provided by testing in compliance with cGMP and EudraLex regulations provides more consistency and confidence for translational groups heading to the clinic. A comparison of the non-cGMP and cGMP compliant services are further described in Table 1.

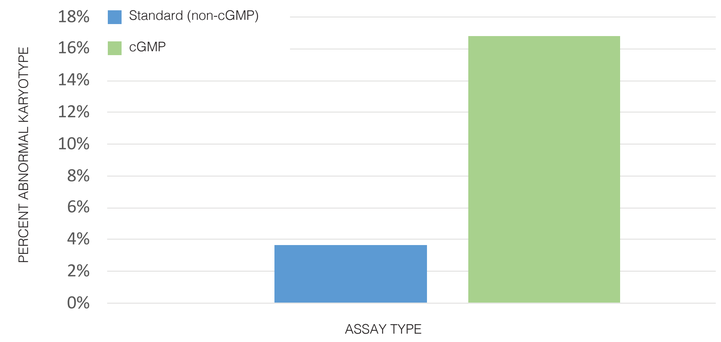
When reviewing the past 5 years of data, the differences seen between the abnormality rate in cGMP compliant testing and standard karyotype testing are substantial. GMP compliant karyotype studies show an abnormality rate of 3.6 percent while over the same period standard karyotype abnormality rate was 16.8 percent (Figure 4). While this is not necessarily surprising, it is notable. Previous reports show that abnormality rate in standard karyotype analysis range between 14-20%. Our findings of 16.8 percent align with those expectations.

FIGURE 2: Growth of GMP Karyotype Assay Numbers



Stem cells are known to acquire genetic mutations in culture. There are numerous consequences from this reality of working with cultured cells and it complicates transitional research and product development (Andrews, et al. 2022). These data show however, that when systems are closely controlled, rates of abnormal karyotype results can be significantly improved. In general, laboratories submitting samples for GMP-compliant analysis are operating under GMP Quality Standards. Cell culture is done in adherence to SOPs, eliminating a significant amount of technician to technician or laboratory to laboratory variability. Equipment is regularly monitored and maintained, reagent quality strictly controlled, physiochemical factors (gas concentration, temperature, etc.) are monitored closely and cell line selection is done carefully. Characterization is performed at specific time points, and materials that do not meet specifications are investigated and frequently discarded. These data demonstrate that these efforts can affect the likelihood of karyotypic changes that impact research and development. This may be important information for regulatory oversight, as the assumption has been that GMP-compliant cell lines have abnormality rates that mirror research lines, when in fact the abnormality rate of cell in controlled culture and research conditions appears to be considerably lower with an improved risk profile.

FIGURE 3: Abnormal Results Rate by Assay Type



SUMMARY

- Use of GMP-compliant assays for genomic analysis in support of translational studies has increased annually since 2020
- GMP-compliant assays include increased documentation, equipment and process controls, and quality oversight as dictated by 21CFR Part 211
- Abnormality rates are significantly reduced in cultures submitted for GMP-compliant testing suggesting that increased control of culture performance, equipment, reagents and materials can dramatically decrease abnormality rates in cell culture
- This finding may impact how we view potential abnormality rates in translational work, and the risk it represents

ACKNOWLEDGEMENTS

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