Comparative Analysis of 1R6F Reference Cigarette in TK6 and CHO cell lines utilizing Flow cytometry and Manual Counting for the *in vitro* Micronucleus Assay

[1]Sean Oh, [1]Katarina Aleksa, [1]Dong Ma, [1]Charmi Mehta, [1]Landen Power, [1]Bonnie Coffa

[1]Labstat International, Kitchener, ON, Canada

Background and purpose

This study aims to compare the responses between manual scoring and automated scoring for Kentucky reference standard 1R6F in two distinct cell lines, TK6 and CHO. Following exposure, underwent in vitro micronucleus testing to assess genotoxic effects. Flow cytometry, a high-throughput technique, and manual slide scoring, a traditional method, were employed as complementary approaches for result validation. Briefly, Kentucky reference standard 1R6F, was smoked using a rotary smoking machine, and the particulate phase (PP) was extracted with DMSO and applied to cells using 5 different concentrations and 3 treatment schedules as per OECD 487. Manual counting, although time consuming, served as a reliable benchmark for validating the flow cytometry results. Proficiency and familiarity with gating principles are essential for obtaining reliable results in MN assay using flow cytometry.

Methods

1) Assay Format

Format	Cell seeding	Data collection
Manual	TK6: 125,000 cells/mL CHO: 100,000 cells/mL	Slide (Microscope)
FC (TK6)	125,000 cells/mL	Tube (Flow cytometry)
High Throughput (CHO)	37,500 cells/mL	HTS (Flow cytometry)

2) Sample extraction - 1R6F

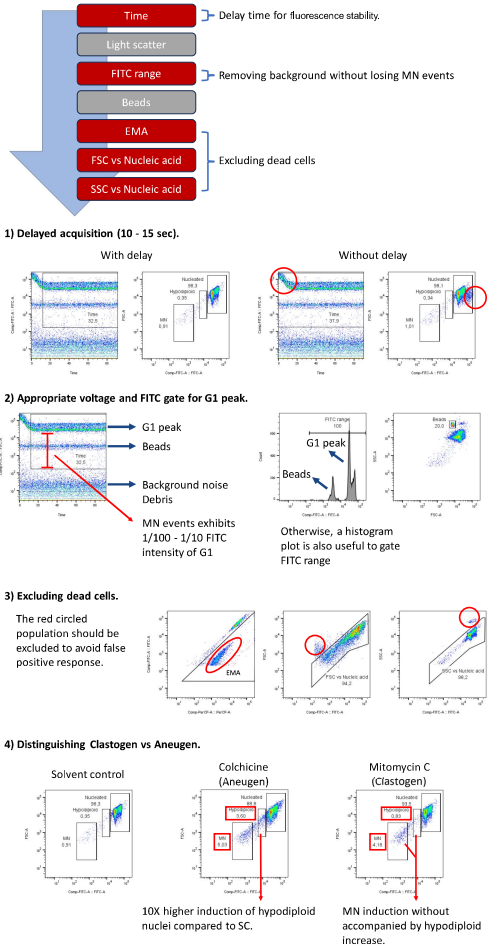
- Cigarette smoked.
- TPM collected on 92 mm filter pad.
- Extracted with DMSO at 10 mg TPM/mL DMSO (stored at -70C).

3) Flow cytometry

- BD FACSLyrics
- Flowjo (FCS raw data analysis)

Results

Figure 1.
Key strategies for Gating of flow MN.



Results

Figure 2.
Manual vs Flow cytometry: Controls

Positive controls of flow cytometry MN induced responses that are compatible with those generated in the manual positive control data base and produce a statistically significant increase compared with the concurrent negative control.

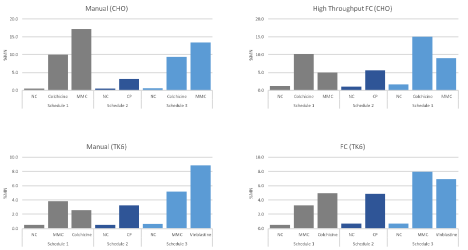
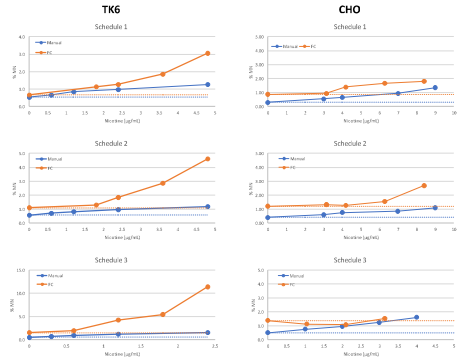


Figure 3.
Manual vs Flow cytometry: 1R6F

The automated MN assay exhibited a higher %MN compared to the manual method in TK6 cells. The dashed lines indicate the %MN of solvent controls for each method (Blue: Manual, Orange: Flow cytometry). The similar pattern was observed for %Cytotoxicity from both manual scoring and flow cytometry (data not shown). As for Schedule 3 in MN CHO cells, the data was not applicable for analysis due to a high percentage of cytotoxicity (OECD 487).



Results

- A precise gating strategy is essential for the MN assay using flow cytometry to prevent false positive or negative responses.
- Manual vs FC - FC detected a higher %MN, while %Cytotoxicity was similar.
- Flow cytometry demonstrates comparable genotoxic conclusions to manual slide counting.
- The flow cytometry MN assay offers a significantly quicker turnaround time compared to manual scoring.
- The efficiency of flow cytometry suggests it as a preferable choice due to its time-saving potential and increased sensitivity.
- Adoption of flow cytometry for micronucleus assay offers a streamlined and reliable alternative to manual counting techniques.

Reference

1. Bryce et al., 2007. "In vitro micronucleus assay scored by flow cytometry provides a comprehensive evaluation of cytogenetic damage and cytotoxicity", Mutation Research. 630(1-2): 78–91.

2. Bryce et al., 2010. "Miniaturized Flow Cytometry-Based CHO-K1 Micronucleus Assay Discriminates Aneugenic and Clastogenic Modes of Action", Environmental and Molecular Mutagenesis. 52:280-286.

3. Avlasevich et al., 2011. "Flow cytometric analysis of micronuclei in mammalian cell cultures: past, present and future", Mutagenesis. 26(1), 147-152.

4. OECD Guideline for the Testing of Chemicals No. 487, 2023. "In Vitro Mammalian Cell Micronucleus Test".

The previous title of the poster was "Comparative Analysis of Heated Tobacco Products and 1R6F Reference Cigarette Responses in TK6 and CHO cell lines utilizing High Throughput Flow cytometry and Manual Counting for the in vitro Micronucleus Assay".