Development of a Bioassay for the Detection of Anti-human Growth Hormone Neutralizing Antibodies using hGHR-expressing BA/F3 Cells
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Purpose
To develop an improved cell-based assay for detecting neutralizing antibodies directed at human growth hormone (hGH) present in human serum samples.

Methods
The assay is based on the inhibition of hGH-induced BA/F3-hGHR cell proliferation by hGH neutralizing antibodies. The BA/F3-hGHR cell line is a murine pro-B cell lymphoma line stably transfected with hGH receptor (hGHR) which proliferates in response to hGH or murine interleukin-3 (mIL-3). Proliferation is measured through ATP quantitation (Promega Cell-Titer Glo). If present, anti-hGH neutralizing antibodies bind hGH and prevent cell proliferation. Neutralization activity is calculated as the relative activity of a given sample to that of the negative control and the subsequent comparison of that relative activity to an established cut point. An alternative inducer (mIL-3) is used to screen samples to detect non-specific inhibition of cell proliferation.

Results
Critical assay parameters were optimized, including cell concentration, cell seeding conditions, assay media components, cell treatment times, and minimum required dilution of samples. Cell treatment times of 24 and 48 hours were compared: the 48 hour treatment produced greater stimulation by hGH as well as greater inhibition by a goat anti-hGH positive control antibody, thereby providing a greater signal-to-noise ratio. Several inducer concentrations between EC10 and EC60 were evaluated and an hGH concentration of 500 pg/mL (~EC40) was selected for the final assay conditions. Under optimized conditions the cut point factor (normalized inhibition) was determined to be 0.71 by testing individual serum samples from 50 normal healthy human subjects. Assay sensitivity was calculated as 250 ng/mL of the goat anti-hGH antibody. The assay has a drug tolerance of up to 10 ng/mL of Genotropin (recombinant hGH). The assay is highly specific to hGH stimulation. Human cytokines/growth factors expected to be present in study samples (IL-2, IL-3, IL-4, IL-6, GM-CSF, M-CSF) showed no interference at physiological concentrations.

Conclusion
The pre-validation data demonstrate that the method is reliable for the detection of anti-hGH neutralizing antibodies in human serum samples.