PharmaSUG 2025- Paper ST-108

Automating Superscript Display for Upper and Lower Limit of Quantification Values in Pharmacodynamic Tables

Anu Eldho, Kite Pharma

ABSTRACT

Pharmacodynamics plays a critical role in clinical trial oncology, as it helps to understand and evaluate the therapeutic effects and mechanisms of action of anticancer drugs. This paper aims to develop a macro that automates an essential and time-consuming part of superscripting the upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) values in the cytokine table outputs. This tool simplifies the task of biostatisticians, pharmacodynamic scientists, and medical writers in superscripting the outputs manually, which is time-consuming and prone to human errors. Scientists can accurately contextualize drug safety and efficacy conclusions by clearly labeling values below or above the validated range. ULOQ, the highest concentration of cytokine values, and LLOQ, the lowest concentration of cytokine values, define the range within which the analytical method can reliably and accurately quantify the concentration of a particular cytokine in a sample. Establishing an appropriate limit of quantification values is crucial in cytokine analysis to maintain the quantification range, data interpretation, dilutional integrity, and quality control. Manual assignment of the superscripts to flag these values is tedious and impractical. This macro helps to compare the actual results values in the data transfer with the cytokine limit of quantification values in the lookup table provided by scientists and flag the consistent values in the TFL outputs. Any updates to a specific cytokine assay will trigger the lookup update, and the programmers can refresh the table outputs without any macro updates.

INTRODUCTION

Cytokines are essential in clinical trials, especially those involving immunotherapies, inflammatory diseases, and certain cancers. Their role is critical in clinical trials, particularly in the areas below.

1. Biomarkers: Clinical trials frequently analyze cytokine concentrations as biomarkers to assess the effectiveness of a therapeutic intervention or to track the body's immune system response. Variation in cytokine levels helps to determine the pathways by which a medicinal agent or therapeutic approach produces its intended effect.

2. Safety monitoring: Monitoring cytokine levels can also help assess a treatment's safety. Certain therapies, such as immunotherapies or cytokine-based therapies, can lead to cytokine release syndrome (CRS) or other adverse events related to cytokine dysregulation. Measuring cytokine levels can help identify and mitigate these side effects.

3. Mechanism of action studies: Clinical trials often include exploratory studies to understand a drug or therapy's mechanism of action. Cytokine analysis in the clinical trials helps to comprehend the immune system's responses and inflammatory processes.

4. Combination therapies: In trials involving combination therapies, cytokine measurements can help understand different treatments' synergistic or additive effects on the immune system or inflammatory processes.

It's important to note that the specific cytokines measured, and their relevance may vary depending on the disease or condition studied and the therapy evaluated. Careful selection of cytokine panels and appropriate time points for measurement is crucial in designing and interpreting clinical trials involving cytokine analysis.

CYTOKINE ULOQ AND LLOQ

The ULOQ and LLOQ values of specific cytokines vary depending on the dilution factor, analytical method (e.g., ELISA, multiplex assays, mass spectrometry-based methods), and sample matrix (e.g., serum, plasma, cell culture supernatant, cerebrospinal fluid supernatant). Proper validation methods and adherence to established guidelines are crucial to ensure reliable and accurate quantification of cytokine levels in various applications.

The ULOQ is the highest cytokine concentration and is quantified with acceptable accuracy, precision, and linearity using the analytical method. It represents the upper limit of the calibration curve, beyond which the quantification may become unreliable due to factors such as saturation of the detection system or non-linear response. The LLOQ is the lowest cytokine concentration and is quantified with acceptable accuracy, precision, and linearity using the analytical method. It represents the lower limit of the calibration curve, below which the quantification may become unreliable due to factors such as ambient noise or interference from the matrix.

Establishing appropriate ULOQ and LLOQ values is crucial in cytokine analysis for the following reasons:

1. Quantification range: The ULOQ and LLOQ define the quantifiable range of the analytical method, ensuring that the measured cytokine concentrations fall within the reliable and accurate range.

2. Dilution integrity: If the cytokine concentrations in a sample exceed the ULOQ, the sample may be diluted based on the vendor and the SOP. In those instances, the ULOQ value helps to determine the appropriate dilution factor to bring the concentration within the quantifiable range. The appropriate dilution factor is determined during assay validation for the in-house cytokine assays.

3. Data interpretation: Accurate quantification of cytokine levels is essential for interpreting biological processes, monitoring disease progression, and evaluating therapeutic responses in various clinical and research settings.

4. Quality control: The quality control samples are tested at or near the LLOQ or ULOQ values to verify that the assay performs reliably within the validated range.

When assigning values to cytokine levels below the lower limit of quantification (LLOQ) and above the upper limit of quantification (ULOQ), it is standard practice to report them as "< LLOQ" and "> ULOQ" respectively, indicating that the measured value falls outside the reliable, quantifiable range of the assay. Depending on the specific analysis method and protocol, some researchers may assign the actual LLOQ and ULOQ values. In our case, a particular threshold numeric value is given when the exact values are not in the quantifiable range. This caused the need to flag these values in the table outputs to differentiate them from the measurable range of values.

Bringing a new biological product from initial research to regulatory approval and commercialization is typically a long and complex journey that can take many years. Clinical trials play a significant role in this journey. It is a lengthy and resource-intensive process, but it ensures that biological products meet stringent safety and efficacy standards before being made available to patients. Clinical trials and analysis follow the study SAP (Statistical Analysis Plan) and Protocol. Development and execution of the clinical trial and subsequent analysis involves cross-functional collaboration of different functions, such as clinical development, statistical programming, biostatistics, clinical pharmacology, regulatory medical writing, etc. The biostatisticians collaborate with the study management team and draft the mock shell for the tables, listings, and figures. Once the TFL shells are approved, the statistical programmers use the mock shell to generate the TFL outputs. The biostatisticians incorporate the outputs in the Clinical Study Report (CSR) or Translational CSR report and submit them to the regulatory authorities for review and approval.

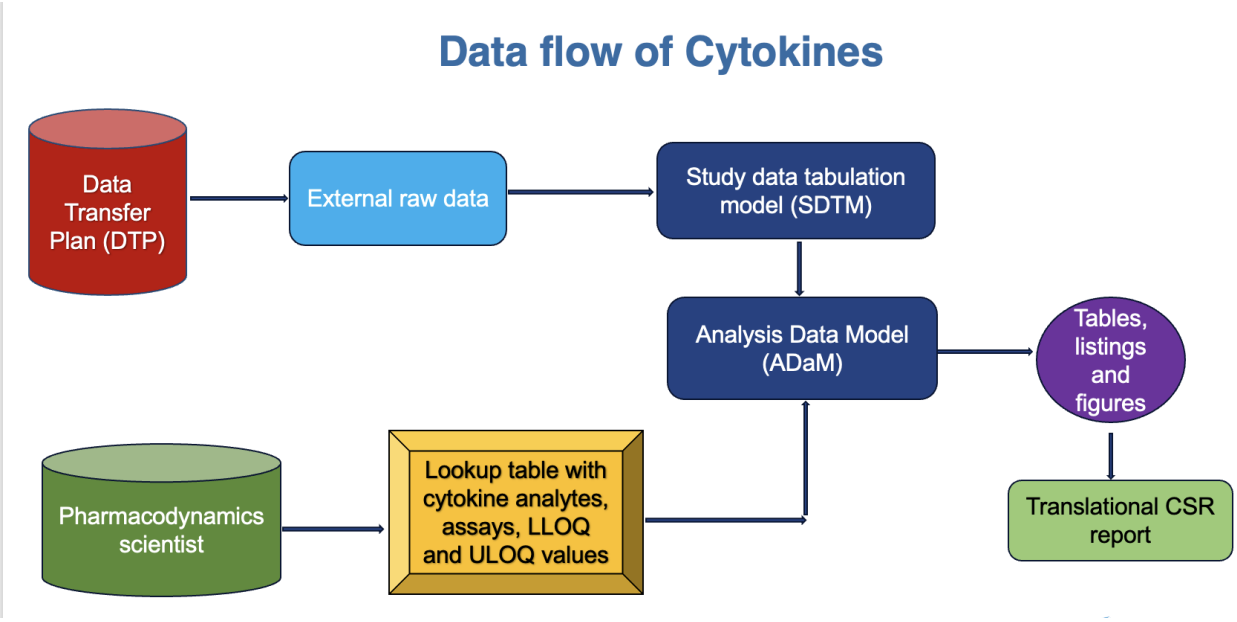
Pharmacodynamics with safety and efficacy outcomes

In most immunotherapy trials, particularly those involving cell therapies, there is interest in analyzing the pharmacodynamic results with the safety and efficacy outcomes. Among the safety outcomes, the highest priority is cytokines associated with neurotoxicity and cytokine release syndrome (CRS). Careful monitoring of these adverse events is needed to ensure patient safety and optimize treatment outcomes. TFLs related to cytokines linked with safety and efficacy outcomes are often of interest to regulatory authorities. Integrating pharmacodynamic data with efficacy outcomes in immunotherapy trials is crucial for elucidating the mechanisms of action, optimizing dosing regimens, identifying predictive biomarkers, guiding combination strategies, and ultimately improving the therapeutic benefit for patients.

Data flow of Cytokines

Cytokines are outsourced to external lab vendors and transferred as external data following a data transfer plan instead of electronic data capture. When the planned cytokine list is finalized for a study, pharmacodynamic scientists will provide the programming team with the lookup table with the list of assays and cytokines with the assigned LLOQ and ULOQ values for serum, plasma, and cerebrospinal fluid (CSF) samples, as applicable. Some analytes in the lookup table require unit conversion in the TFLS. These analytes' LLOQ and ULOQ values are converted in the lookup table using the conversion factor based on the scientist's request, and separate parameter codes differentiate between original and converted units. Once the raw data has passed the reconciliation checks, it is then processed to map it to a custom study data tabulation model (SDTM), followed by an analysis data model (ADaM). ADaM uses a lookup table and SDTM domain, and separate parameter codes differentiate between original and converted units.

Figure 1. Data flow of cytokines



OVERVIEW OF MACRO

Initially, the programming team implemented the macro mcytloq_stat as the validation tool for the scientist-annotated cytokine table outputs in the translational CSR reports. Later, it is adapted as the primary tool to populate superscripts for the relevant table outputs as identified in the shell. The macro uses the cytokine analysis data model dataset and the lookup table as the input. The factor variable from the lookup table is used as a conversion factor in the ADaM dataset to handle the converted units of

analytes. This macro generates summary statistics that fit the standard cytokine summary tables and the LOQ indicator. The macro must be called outside the data step for the use as per the requirement.

Process flow

The macro uses the cytokine ADaM dataset and the lookup table as the input. The lookup table is provided to the programming team by the Pharmacodynamics scientist. The lookup table contains the list of cytokine analytes, assay number, the dilution factor, and the LLOQ and ULOQ variables. The input cytokine dataset contains one record per subject per parameter per each "byvars" variable. If the results in the ADaM dataset match the LLOQ or ULOQ values in the lookup table, the output datasets are generated with the LLOQ and ULOQ flags. The macro generates the summary statistics, which can merge with the datasets with the LLOQ and ULOQ flags. Specific precision rules are applied to the summary statistics to ensure the statistics data meets specific criteria or standards provided by the scientists.

Special conditions apply to display superscripts for only min, max, and median values when the values are ULOQ and LLOQ, as well as to exclude the derived values and statistics like n and standard deviations. If the actual min values or max values match the assigned LLOQ or the assigned ULOQ values from the lookup, those values will be superscripted. If the median values are equal to the 'min' values, which are superscripted, or if the median values are equal to the 'max' values, which are superscripted, then the median values will also be superscripted. Any other summary statistics are excluded from superscripting. The macro has a parameter to control the decimal display in the outputs. By preprocessing the input dataset and passing the right group of by variables in the 'byvars' macro parameter, the source programmer can use the macro for tables with multiple layouts. The Printissues parameter in the macro prints warning messages when the actual result values fall below LLOQ or above ULOQ values. Specific footnotes in the rtf outputs indicate that the reported values represent an assigned numeric value that fell outside the corrected limit of quantification.

Macro call:

```
%macro mcytloq_stat (
                  indata                          = adam
                , avalvar                         = aval
                , byvars                           = avisit colhead
                , ndec                             = 1
                , outdata                          = cyto_stat_lloq
                , out_stat_var                     = median1
                , LoqData                          = lookup.cyt_loq
                , LloqVar                          = loqalt
                , UloqVar                          = loqagt
                , LOQIndChar                       = Y
                , PrintIssues                      = Y
                 );
```

Macro parameters description

Parameters: Name	Required (Y/N)	Description
INDATA	Y	Name of the input cytokine dataset, expected to be one record per subject per parameter and other “byvars” variables

AVALVAR	Y	Name of analysis variable
BYVARS	N	The list of by variables in addition to AVISIT and the PARAMCD. This is an optional parameter
NDEC	Y	Controls the decimal display in the outputs
OUTDATA	Y	Name of the output SAS dataset that contains the summary statistics
OUT_STAT_VAR	Y	Name of the SAS variable that contains the Median (n, Min, Max) values.
LOQDATA	Y	Name of the LOQ lookup dataset along with the library name
LLOQVAR	Y	Variable that contains LLOQ values in the lookup table
ULOQVAR	Y	Variable that contains ULOQ values in the lookup table
LOQINDCHAR	Y	A character indicator for the LOQ values. This can be the STAT specified character in the TFL shell.
PRINTISSUES	N	Print issues in data with LLOQ/ULOQ values

Algorithm in a nutshell

This macro call uses the input ADaM dataset to calculate the summary statistics for the analysis result variable, using the by-group variables like parameter code, analysis visit, column header, and other subgroups as included in 'byvars,' and the variable in the 'outdata' macro parameter captures the result. When the values are LLOQ or ULOQ, the macro adds the superscript 'a' as specified in the 'loqindchar' parameter. The macro can also check and print messages if the results fall below LLOQ or above ULOQ values.

Sample outputs

Figure 2. Superscript display at dataset level

avisit	paramcd	colhead	median1
DAY X	TNFB	FL	1.34 (60,1.34,1.34)
DAY Y	VCAM1CU	FL	222.25 (52, 0.08,8000.82 ^{super a})
DAY Z	AMYLOIDA	CLL	4.327+07 (63, 6.21E+06, 1.58E+10 ^{super a})
DAY W	CCL17	CLL	700.40 (50, 60.90, 5380.00 ^{super a})

The above snippet shows the superscript display at the dataset level inside the table program. Here, the median1 column displays statistics as median (n, min, max) with the superscript ^ {super a} as applicable. The source programmer outputs the dataset for validation purposes. The validation programmer uses the validation macro to compare the table dataset used as the input for rtf output.

Superscript display at RTF level

Figure 3. Cytokine with safety outcomes

Analyte (Post-treatment)	Grade XX or Higher (N= 2) Median (n, Min, Max)	Grade XX, Grade XX, or None (N= 3) Median (n, Min, Max)	Median Ratio
CRP (unit)	2.0 (1,3.14E-08[a],12.0)	4.0 (1, 9.14E-08, 11.0)	2.02
CXCL10 (unit)	300.0[a] (1, 300.0[a], 300.0[a])	300.0[a] (2,300.0[a],300.0[a])	1.09

Abbreviations: AUC, area-under-the-curve; CRP, C-reactive protein; CXCL, C-X-C motif chemokine; GM-CSF, granulocyte-macrophage colony-stimulating factor:

[a] Reported values represent an assigned numerical value given to results that fell outside the dilution-corrected limit of quantification.

Figure 3 is an example of a Pharmacodynamic table with safety outcomes. The table output's footnote has the description of the yellow highlighted superscripts. Based on the requirement, the macro displays superscript to specific subgroups. The subgroup variables can be added in the "by vars" macro parameter to get the expected results.

Figure 4. Cytokine with efficacy outcomes

	Serum Cytokine			
Analyte	Responder	Nonresponder	Median Ratio	P-value
Median (n, Min, Max)	(N = 100)	(N = 1)		
CRP (unit)				
Peak	200 (100, 3.2, 900.0[a])	300.1 (1, 300.1, 300.1)	1.2	0.0121
AUC (unit)*days	200 (100, 3.2, 900.0[a])	400.1 (1, 400.1, 400.1)	2.2	0.0123
Baseline	1.2 (100, 10.0, 900.0[a])	2.1 (1, 2.1, 50.0)	2.2	0.0121
Day X	1.2 (100, 10.0, 900.0[a])	2.1 (1, 2.1, 50.0)	1.2	0.0123
Day Y	1.2 (100, 10.0, 900.0[a])	2.1 (1, 2.1, 50.0)	1.2	0.0121

Figure 5. Cytokine overtime table

Analyte	Baseline Median (n, Min, Max)	Day X Median (n, Min, Max)	Day Y Median (n, Min, Max)	Day Z Median (n, Min, Max)	Week X Median (n, Min, Max)	Week Y Median (n, Min, Max)	Peak Median (n, Min, Max)	AUC Median (n, Min, Max)
IL-7 (unit)	3.0 (100, 2.0[a], 60.0)	2.5 (100, 2.0[a], 60.0)	2.5 (100, 2.0[a], 60.0)	2.5 (100, 2.0[a], 60.0)	2.5 (98, 3.0,80.0)	2.5 (98, 3.0,80.0)	2.5 (100, 5.0, 100.0)	2.5 (100, 5.0, 100.0)
IL-8 (unit)	4.0 (100, 3.0[a], 200.0)	4.0 (100, 3.0[a], 200.0)	4.0 (100, 3.0[a], 200.0)	4.0 (100, 3.0[a], 200.0)	4.0 (98, 3.0[a], 100.0)	4.0 (98, 3.0[a], 100.0)	4.0 (98, 3.0[a], 100.0)	4.0 (98, 3.0[a], 100.0)

Figure 5 is an example of a Pharmacodynamic table over time in horizontal format. The macro fits multiple formats. Data preparation before calling the macro is needed to get the expected results.

CONCLUSION

To summarize, macros can be used in TFL programs to generate repetitive code patterns automatically. It's worthwhile to summarize macros' capabilities and emphasize their main advantages. Macros can create the code for the equations, reducing the effort required for manual coding and minimizing the risk of errors. They can also conditionally include or exclude code segments based on specific requirements or options. They can help generate TFLs with different complexity levels or turn certain features on/off.

The macro described in this paper allows the programming team to generate TFL outputs with superscripts without added complexity. This macro is designed to be used across studies in the table programs without major updates for the same data type.

REFERENCES

Cytokines in clinical cancer immunotherapy, Pedro Berraondo, Miguel F. Sanmamed, María C Ochoa Iñaki Etxeberria, Maria A. Aznar, José Luis Pérez-Gracia, María E. Rodríguez-Ruiz, Mariano Ponz-Sarvise, Eduardo Castañón & Ignacio Melero.

Clinical Application of Cytokines in Cancer Immunotherapy, Yi Qiu, Mengxi Su, Leyi Liu, Yiqi Tang, Yuan Pan, Jianbo Sun.

Carmeli, I. and Bar, Y., (2022), Validation of Statistical Outputs Using Automation, Proceedings of the PharmaSUG 2022, Paper SA-011.

Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays, Wilco de Jager, Katarzyna Bourcier, Ger T Rijkers, Berent J Prakken, Vicki Seyfert-Margolis.

ACKNOWLEDGMENTS

I would like to sincerely thank Rajesh Venkkataram and Madison Davis for their invaluable guidance, insightful feedback, and continuous encouragement throughout the course of this work. I extend my appreciation to my colleagues in the translational programming team for their collaborative efforts and support. I am also thankful to the upper management of Kite Pharma for their backing of this endeavor.

CONTACT INFORMATION

Your comments and questions are valued and encouraged. Please feel free to contact the author at:

Anu Eldho
Kite Pharma
anu.eldho@gilead.com