

Multifunctional SAS Macro for Fold Rise Calculation

in Immunogenicity Analysis

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ABSTRACT

The Center for Biologics Evaluation and Research (CBER) and European Medicines Evaluation Agency (EMA) have different criteria for calculating immunogenicity fold rise when serology data contains Lower Limit of Quantification (LLOQ). This paper introduces a multifunctional SAS macro that is capable of calculating the fold rise per both criteria with the flexibility to calculate fold rise across different visits and in the meantime capturing the LLOQs of multiple antibody strains.

KEY WORDS

Fold Rise, CBER, EMA, LLOQ, ULOQ, Immunogenicity

INTRODUCTION

In simple sense, the fold rise is calculated as the ratio of the post-vaccination titer value to the pre-vaccination value. The geometric mean of the fold rise is called Geometric Mean Fold Rise (GMFR). In vaccine studies, however, LLOQ (Lower Limit of Quantification) and ULOQ (Upper Limit of Quantification) are very common for antibody titers, especially the LLOQ case. The values of LLOQ and ULOQ vary with different antibody strains. In practice, the >ULOQ case usually uses the ULOQ as the substitute for the missing value. The <LLOQ case, however, is handled differently per different regulator criteria.

Figure 1: A Simple Numeric Illustration of EMA and CBER criteria

					For Fold-rise and GMTR calculation					
	Titers		For GM calculation		CBER			EMA		
Subject	Pre	Post	Pre	Post	Pre	Post	Fold Rise	Pre	Post	Fold Rise
1	<10	<10	5	5	10	5	0.5	5	5	1
2	<10	10	5	10	10	10	1	5	10	2
3	10	<10	10	5	10	5	0.5	10	5	0.5
4	10	10	10	10	10	10	1	10	10	1
5	<10	20	5	20	10	20	2	5	20	4
6	10	40	10	40	10	40	4	10	40	4

For the calculation of regular Geometric Means of Titers and seroprotection, <LLOQ is converted to 0.5*LLOQ per both CBER and EMA criteria. Things become different when it comes to calculate fold rise. When the pre vaccination value is < LLOQ and the post value is at or above LLOQ, the CBER criterion converts the < LLOQ to LLOQ but EMA

uses $0.5 \times \text{LLOQ}$. When the pre vaccination is at or above LLOQ and post vaccination value is $< \text{LLOQ}$, both criteria converts the $< \text{LLOQ}$ to $0.5 \times \text{LLOQ}$. When both the pre and post values are $< \text{LLOQ}$, the fold rise is set to 1 per both criteria. In general, the CBER criterion is more conservative and more difficult to get higher fold rise values. Figure 1 is a simple numeric illustration of the difference between EMEA and CBER criteria.

Figure 2: Example of Fold Rise across Different Visits

			PPrV 10 µg+adj (N=#####)			PPrV 25 µg+adj (N=#####)			PPrV 50 µg+adj (N=#####)			Placeb (N=#####)	
			n/M	%	(95% CI)	n/M	%	(95% CI)	n/M	%	(95% CI)	n/M	%
anti-PpA IgG (ELISA-EU/mL)	Post-Injection 2/pre-Injection 1	≥ 2 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
		≥ 4 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
	Post-Injection 3/pre-Injection 1	≥ 2 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
		≥ 4 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
	Post-Injection 3/post-Injection 2	≥ 2 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
		≥ 4 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
anti-PhtD IgG (ELISA-EU/mL)	Post-Injection 2/pre-Injection 1	≥ 2 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
		≥ 4 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
	Post-Injection 3/pre-Injection 1	≥ 2 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
		≥ 4 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
	Post-Injection 3/post-Injection 2	≥ 2 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
		≥ 4 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
anti-PlyD1 IgG (ELISA-EU/mL)	Post-Injection 2/pre-Injection 1	≥ 2 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
		≥ 4 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
	Post-Injection 3/pre-Injection 1	≥ 2 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
		≥ 4 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
	Post-Injection 3/post-Injection 2	≥ 2 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %
		≥ 4 fold-rise	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %	(### %, ### %)	#####	### %

THE FOLD RISE MACRO

The following SAS macro is developed to calculate fold rise in according to the different regulator criteria specified in the previous section. Five macro parameters are created in this macro. The *dsin* parameter specifies the name of the input data set. The *testn* parameter is the total number of strains that need to calculate fold rise. The *visit* parameter is used to list all the visitnumbers involved for fold rise calculation in an ascending order. The *sf* parameter is for suffix. Sometimes in immune-analysis, fold rise needs to be calculated not only for the ratio of the last and first blood sample results, but also across other visits, for example, the last and the last but one visit. Figure 2 is an example of fold rise across different visits. The *visit* and *sf* parameter of this macro are designed to deal with such situation, which offers the flexibility to calculate fold rise across all the visits listed in the *visit* parameter.

```
%macro foldrise(dsin=, testn=, visit=);
proc sql noprint;
    select distinct paramn into :lloqn separated by ' '
    from &dsin where index(lborres, '<');
quit;

%do n=1 %to &testn;
%if %sysfunc(indexw(&lloqn, &n)) %then %do;
    proc sql noprint;
        select distinct compress(lborres, '<') into :lloq&n
```

```

        from &dsin. where paramn=&n & index(lborres,'<');
quit;
%end;
%else %do;
    %let lloq&n=.;
%end;
%end;

%let num=%sysfunc(countw(&visit));
%do v=1 %to %eval(&num-1);
%let sf=%scan(&visit,&v);

proc sort data=&dsin.;by usubjid paramn visitnum; run;

data base&sf.(rename=(aval=abase&sf.) keep=usubjid paramn aval);
    set &dsin.(where=(visitnum=&sf.) );
proc sort;by usubjid paramn;
run;

data &dsin;
    merge &dsin. base&sf.;
    by usubjid paramn;
run;

data &dsin.;
    set &dsin.;
    %do m=1 %to &testn.;
        if paramn=&m then lloq&m=&lloq&m.;
        if visitnum>&sf. & paramn=&m. then do;
            if .<abase&sf.<&lloq&m. & .< aval <&lloq&m. then do;
                IMFOLDCO&sf.=1; IMFOLD&sf.=1;
            end;
            if .<abase&sf.<&lloq&m. & aval>=&lloq&m. then do;
                IMFOLDCO&sf.= aval /&lloq&m.;
                IMFOLD&sf.= aval /abase&sf.;
            end;
            if abase&sf.>=&lloq&m. & aval >=&lloq&m. then do;
                IMFOLDCO&sf.= aval /abase&sf.;
                IMFOLD&sf.= aval /abase&sf.;
            end;
            if abase&sf.>=&lloq&m. & .< aval <&lloq&m. then do;
                IMFOLDCO&sf.=0.5*&lloq&m/abase&sf.;
                IMFOLD&sf. = aval /abase&sf.;
            end;
        end;
    end;

```

```

        end;
    %end;
run;
%end;
%mend;

```

IMFOLD and IMFOLDCO variables are fold rise by EMEA and CBER criteria respectively. The value of LLOQ for each test varies. At the beginning of this macro, the LLOQs of each strain are collected and stored in a string. For those serology tests that don't have a LLOQ, its LLOQ value is set to null. Capturing these LLOQs is very useful because they are also used for seroprotection and booster response rate calculation.

CONCLUSION

Fold rise is a key statistics of immunogenicity analysis in vaccine clinical studies. The SAS macro introduced in this paper simultaneously calculates the fold rise in according to both EMEA and CBER criteria. In the meantime, this macro can calculate fold rise for all the visits listed in the macro parameter, which in turn offers the flexibility to calculate fold rise across different visits. In addition, this macro can also capture the LLOQs of multiple antibody strains, thus facilitate vaccine clinical programming.

CONTACT INFORMATION

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