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Accessible, adaptable and automated:
having it all with an immunogenicity cut point calculator

Samuel Pine, EBF 14th Open Symposium

24-26 November 2021

Outline

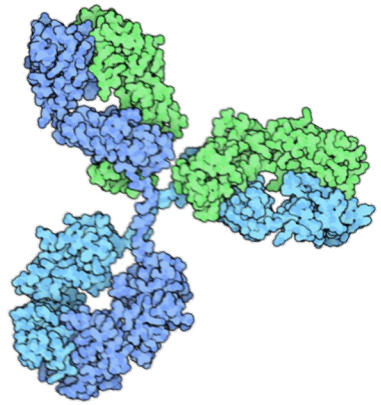
- **Introduction**
  - Statistical requirements for immunogenicity testing
  - Common approaches
- **Design of the *ImmunoStat Simple* cut point calculator**
  - User requirements
  - Technical challenges
  - Global GxP implementation
- **Example ADA cut point analysis and reporting**

Presented at EBF Open Symposium Nov 24-26, 2021

Why do we need immunogenicity testing?

Safety & regulatory requirements for biologics

- **European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP)**
  - Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins (2017). EMEA/CHMP/BMWP/14327/2006 Rev.1.
- **US Department of Health and Human Services, US FDA, Center for Drug Evaluation and Research (CDER), Biologics Evaluation and Research (CBER)**
  - Guidance for Industry, Immunogenicity Assessment for Therapeutic Protein Products (2014).
  - *Guidance* for Industry, Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products (2019)

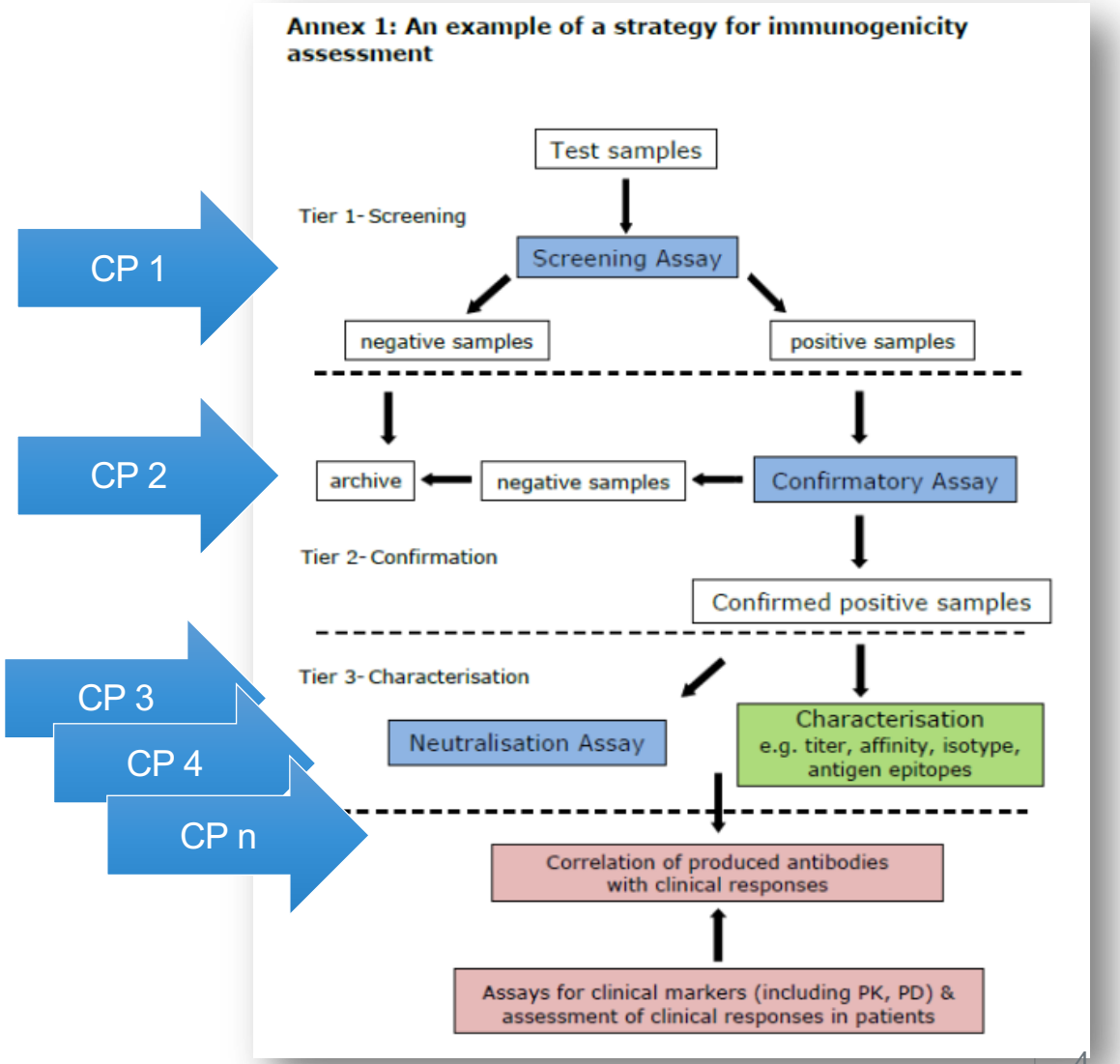


- Affect safety and efficacy
  - Neutralize drug effects
  - Reduce or increase drug exposure
  - Cause serious acute reactions, e.g. anaphylaxis
  - Can cause autoimmunity, e.g. endogenous counterpart
- Immunogenicity ≈ ADA

Semi-Quantitative Testing Strategy

Tiered approach with multiple cut points

- **Screening assay**
  - all samples, determine ability to bind to antigen (drug)
- **Confirmation assay**
  - screen (+) samples, determination of specificity
- **Characterization assays**
  - Titer
  - Neutralizing capacity
  - Domain specificity, isotype, etc.

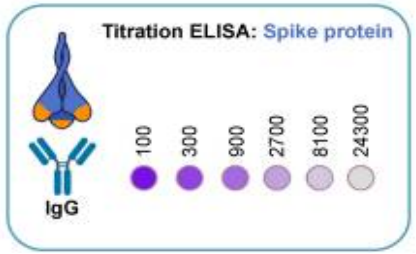


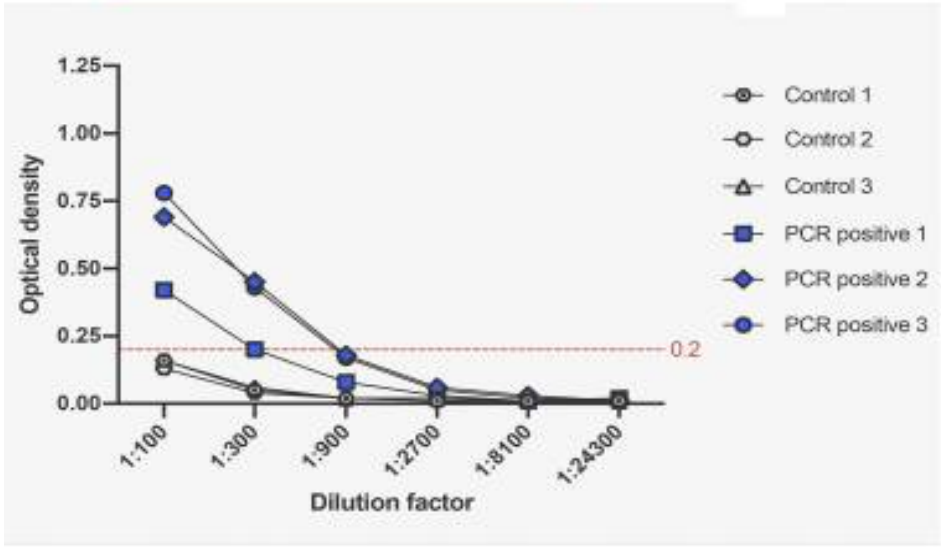
Bioanalysis + Biostatistics

Statistical justification for ADA positivity status

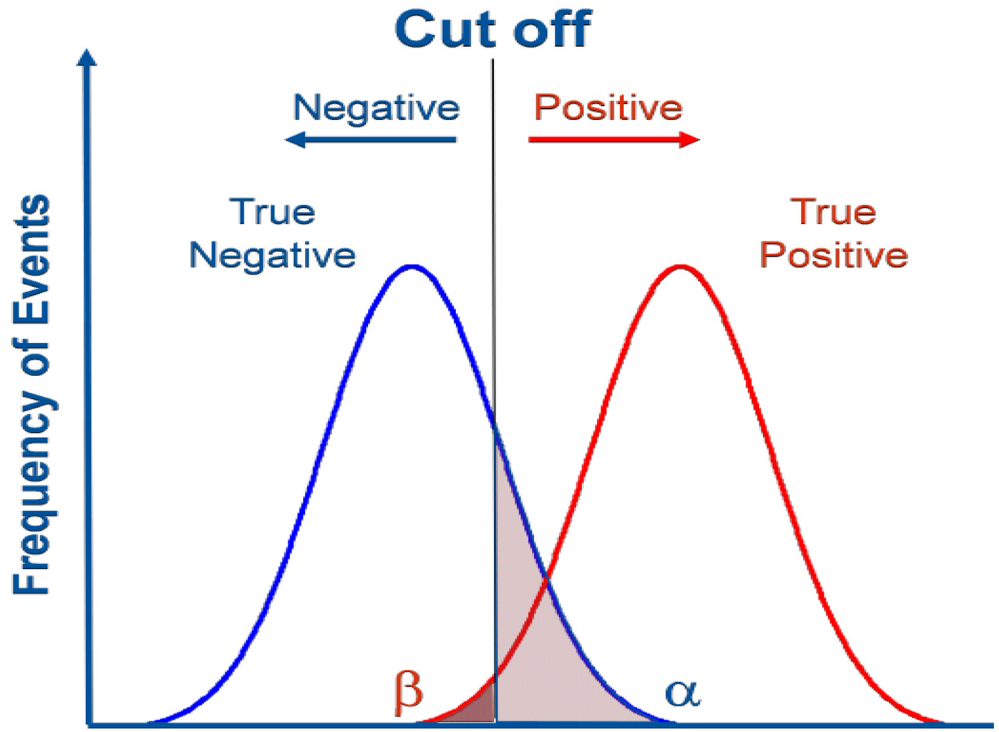
Cut-points (CP) or cut point factors are determined to discriminate positive vs. negative samples

Determined on ‘blank’ or ‘normal’ population, which can contain some level of reactivity





MacGregor et al 2020, *Peer J.*



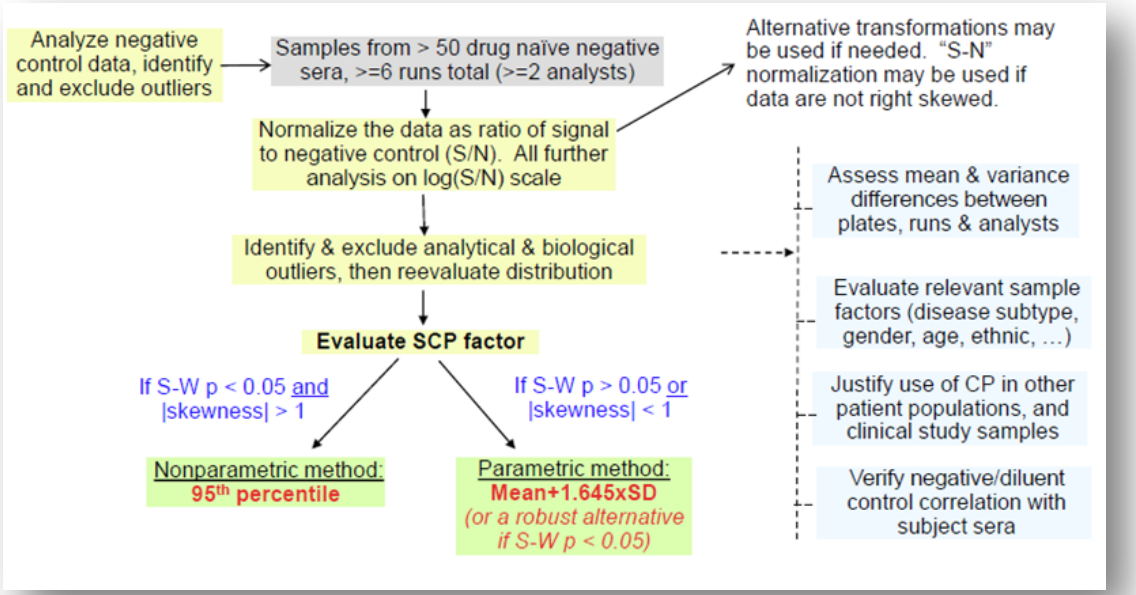
Zhang et al 2020, *J Immunol Methods*

Statistically sound cut point calculations

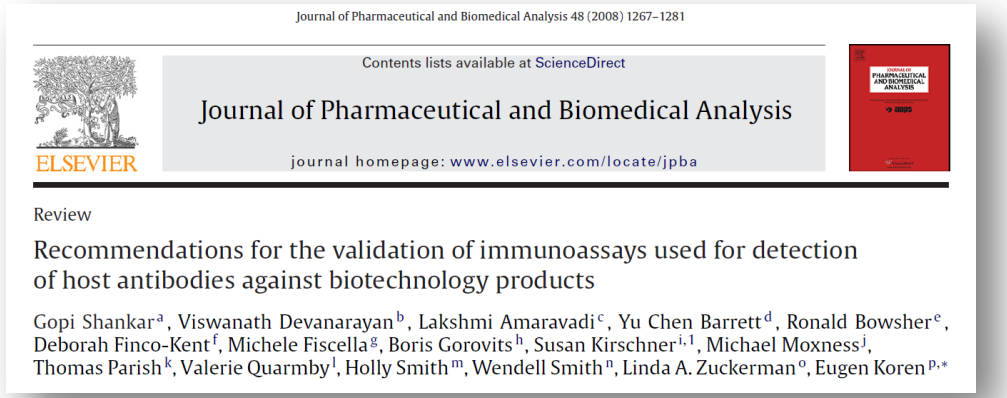
Rigor often adds complexity

Cut-points (CP) or cut point factors are determined to discriminate positive vs. negative samples

Determined on 'blank' or 'normal' population, which often contains some level of reactivity



Devanarayan, V. EIP 2019



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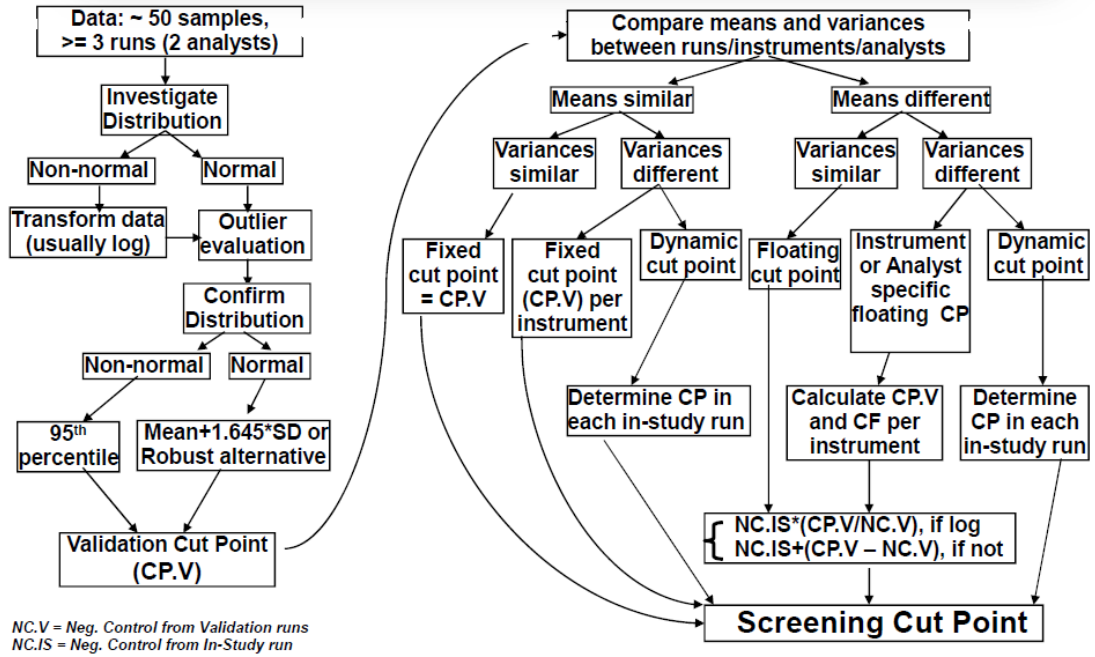
Review

Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products

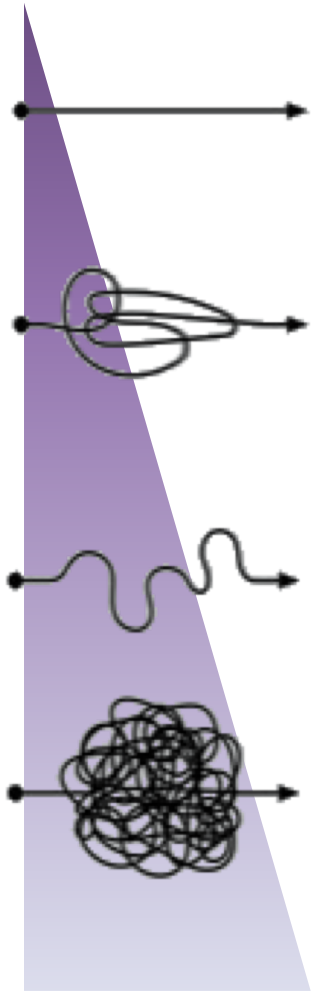
Gopi Shankar[a], Viswanath Devanarayan[b], Lakshmi Amaravadi[c], Yu Chen Barrett[d], Ronald Bowsher[e], Deborah Finco-Kent[f], Michele Fiscella[g], Boris Gorovits[h], Susan Kirschner[i,1], Michael Moxness[j], Thomas Parish[k], Valerie Quarmby[l], Holly Smith[m], Wendell Smith[n], Linda A. Zuckerman[o], Eugen Koren[p,*]



Calculating cut points in practice

Operational hurdles during bioanalytical studies

- Cut points calculated during method validation/qualification as well as in-study
- **Can create a pinch-point**, as CP runs completed first and CP subsequently needed to complete other validation parameters
- Increasingly advanced and complex calculations required
- **Multi-disciplinary** experts or multiple subject matter experts needed
- Although XLS options are available, dedicated statistical software and expertise is preferred
- Passing off **GxP data** from BA lab to Biostatistician requires additional measures to **ensure data integrity**
  - Quality control steps
  - Validation of GxP processes
  - Additional time & resources
- **Slows down** validation/qualifications and **introduces operational complexity**



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Statistical software for bioanalysts

Bridging the gap

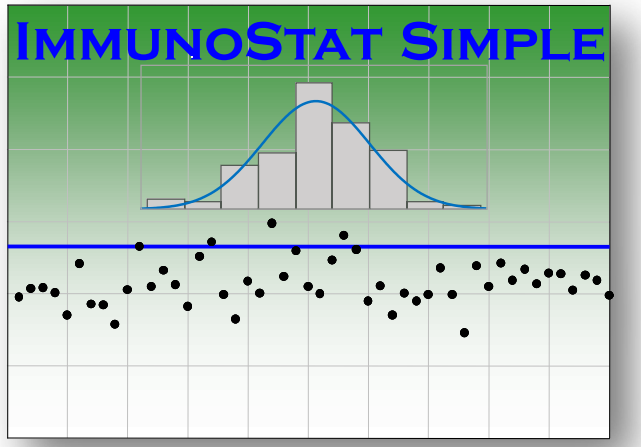
Statistical Discovery.™ From SAS.

- JMP software a common choice for bioanalytical labs: GUI, SAS-based, 21 CFR Part 11 compliance
- Ablynx scientists took note of scriptable functions to automate cut point calculations and reporting
- Script and validation plan originally for one site, post-acquisition scope broadened for five global sites + CROs
- Appropriate globally-accessible GxP environment for JMP implemented e.g., server-based licenses or virtual desktop environment
- Global validation plan executed & system release with implementations using local lab regulations

ImmunoStat Simple

Automated Immunogenicity cut point Calculator

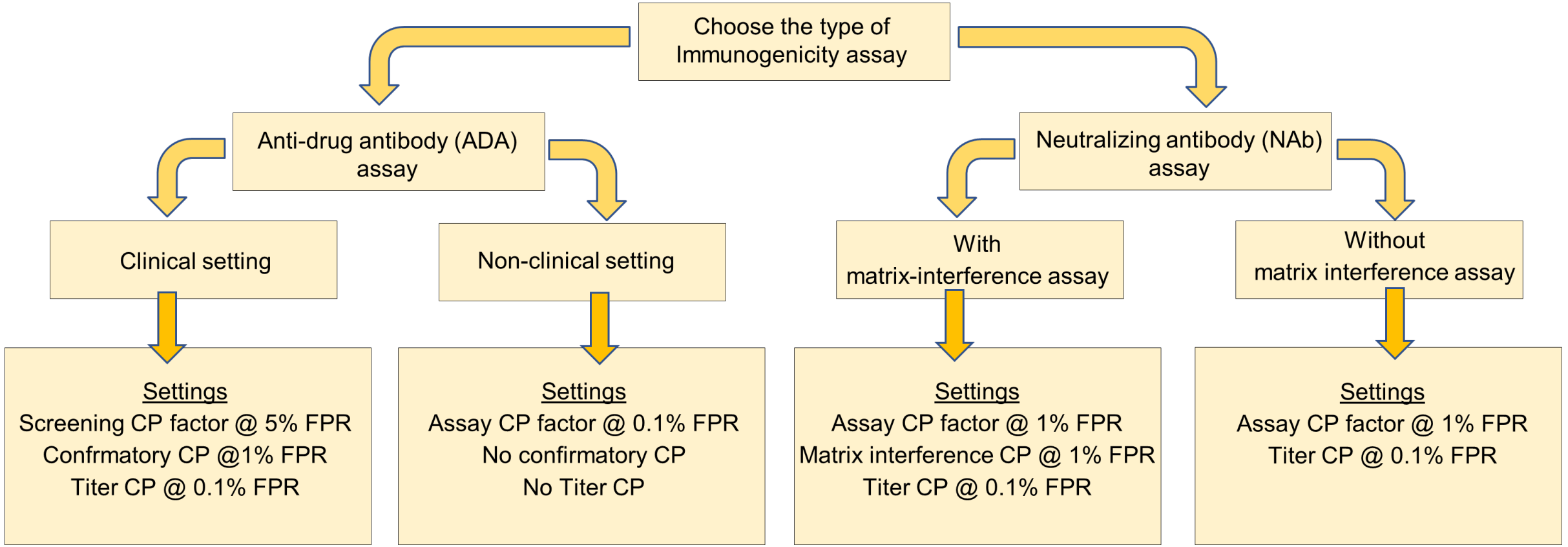
- A validated JMP script used for calculating and reporting immunogenicity cut points



- Scope
  - Screening, confirmatory and titer cut points
  - Nonclinical and clinical ADA and NAb assays
  - Various NAb formats
- Harmonized standard approach for consistent best-practice analysis
- Applicable to internal labs and available for external partners
- Flexible settings allowed to include seldom-used parameters or alternative approaches for specific situations

Overview of CP calculation algorithm

Standardized approach with flexibility built in

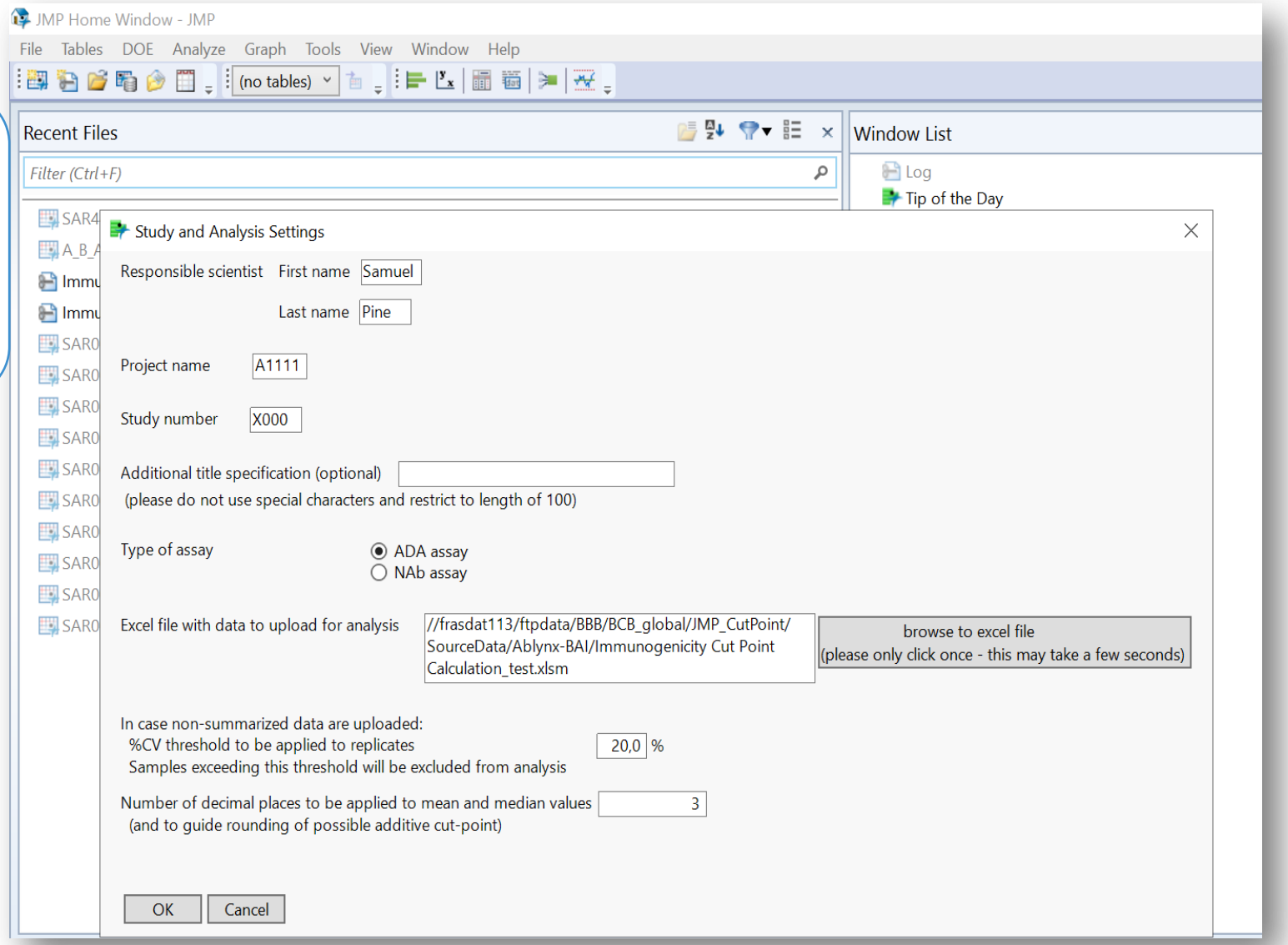


Example Use - Starting up

Example dataset:
- Human ADA assay qualification
- Standard clinical approach
- Selected extra figures/analyses
- 4 runs, n=36 individuals, 2 operators
- Balanced design on multiple assay days

Clinical setting

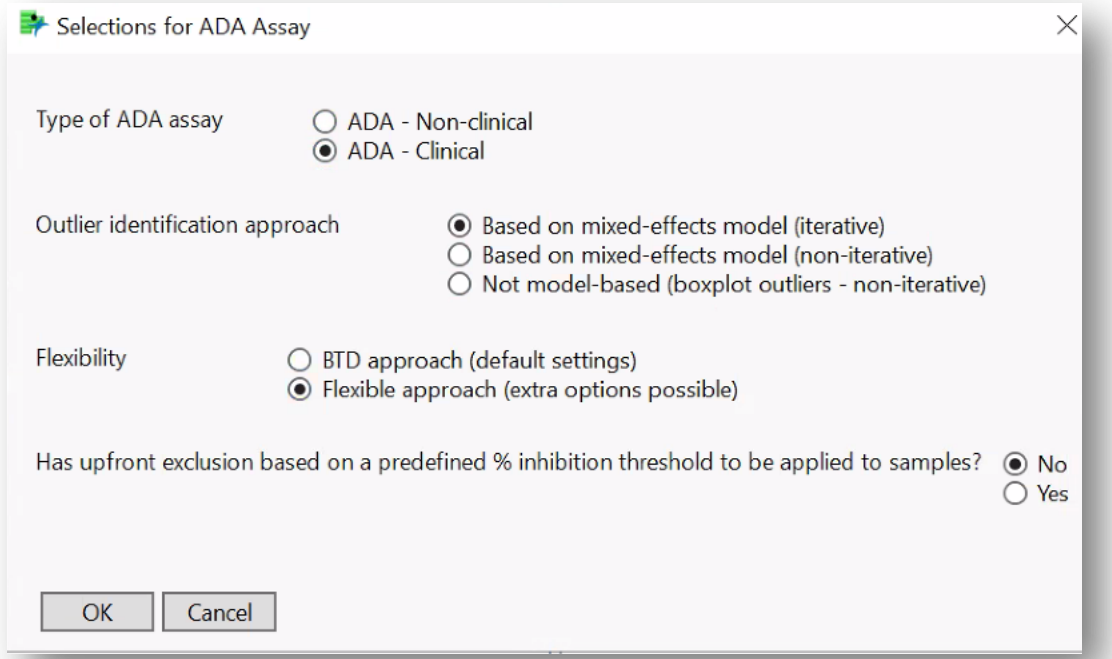
<u>Settings</u>
Screening CP factor @ 5% FPR
Confrmatory CP @1% FPR
Titer CP @ 0.1% FPR

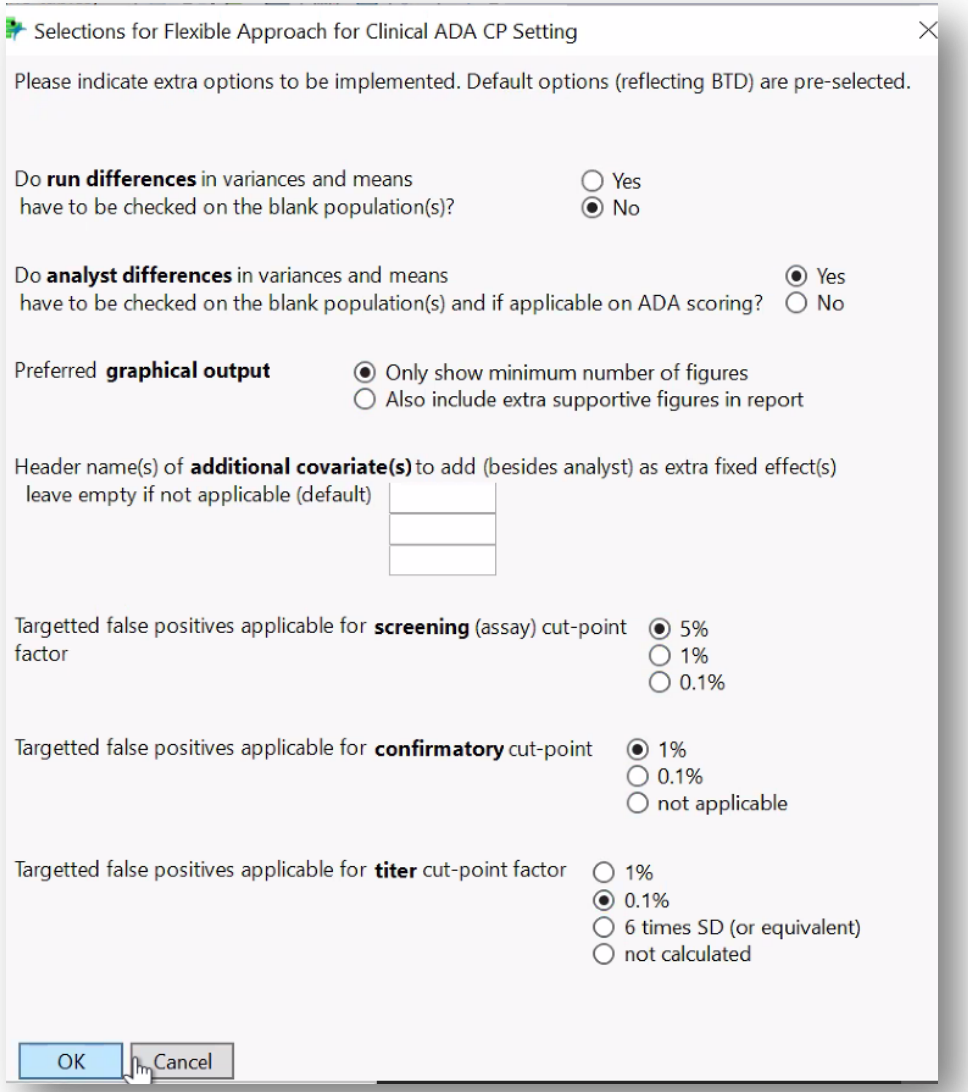


Test selections

Dynamic menus & options rely on previous selections
Standard approach analyses chosen:

- Mixed-effects, iterative outlier removal
- Flexible approach could include run, analyst and other covariates; adjusted FPR rates for each tier





Example Use - Starting up

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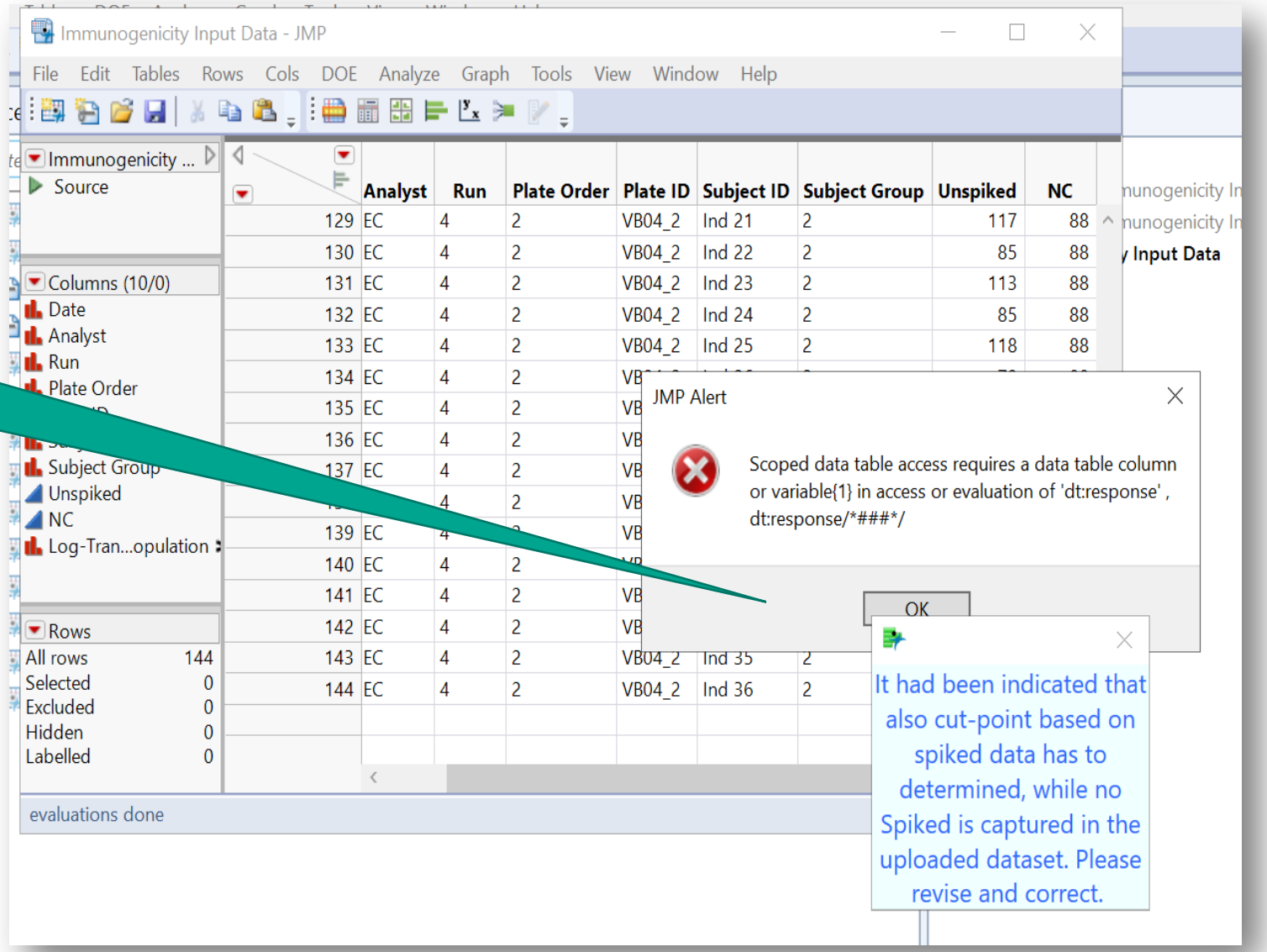
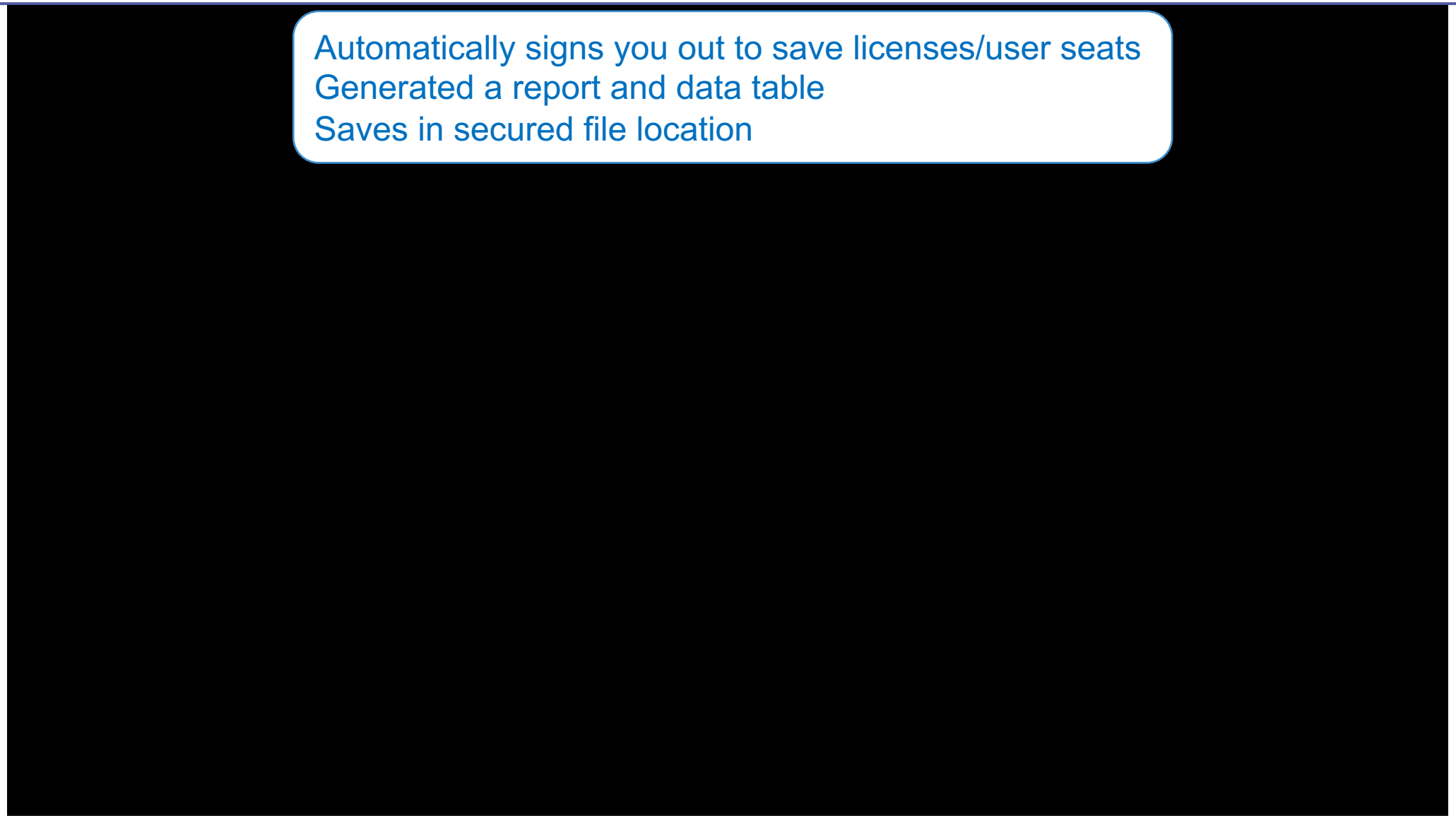
Includes data checks and acceptance testing!



Video Demo



4x video – total elapsed time 1:40

Reporting Output 1

Settings & methodologies

1. Analysis Settings

1.1. System Settings

	System Values
Script version	Immunogenicity CP analysis_v01
Invoked from	//frasdat113/ftpdata/BBB/BCB_global/JMP_CutPoint/Script/Prod/
By (username)	I0407086
From (computername)	DESKTOP-PIQ1C87
On (date and time)	26Oct2021:10:38:27
Uploaded Excel file	//frasdat113/ftpdata/BBB/BCB_global/JMP_CutPoint/SourceData/Ablynx-BAI/Immunogenicity Cut Point Calculation_test2.xlsm

1.2. Selected Options

ADA Cut-Point Analysis	Selected Options for Study
Optional title specification	
Responsible scientist	Pine SAmuel
Assay type	Clinical ADA assay
Confirmatory data	Spiked data included in uploaded dataset
Upfront exclusion	No upfront exclusion of samples performed
Outlier removal approach	Based on mixed-effects model (iterative approach) including {:Analyst} as fixed effect and {:Plate ID[:Analyst,:Run], :Run[:An
Flexibility	Flexible approach
Data upload	Uploaded data already summarized over replicates
%CV threshold	No %CV check performed (no replicates in uploaded dataset)
Number of decimal places	Entered number of decimal places to guide precision for possible additive cut-point factor : 3

Description	Selected Option for Flexible Approach
Analysis for run-specific differences in variances and means on the analysis population(s)	Run differences assessed
Analysis for analyst-specific differences in variances and means on the blank population(s)	Operator differences assessed
Preferred graphical output	Extra supportive figures and tables included in report
Header name(s) of additional covariate(s) to add (besides analyst) as extra fixed effect's	{}

Cut-Point (Factor)	Targetted FPR
Screening cut-point factor	5% FPR
Confirmatory cut-point	1% FPR
Titer cut-point factor	0.1% FPR

All relevant data paths & analysis info captured
Dynamic text changes depending on CP settings

2. Methodology

The cut-point (CP) analysis is based on guidelines described in BTD-010945, RDBTD-002228, RDBTD-002001, RDBTD-002002 and RDBTD-002217.

In order to accommodate to putative plate/run drifts, a floating screening cut-point factor (SCPF) is settled. Therefore, log-transformed ratios of unspiked values divided by their respective negative control (NC) values and unspiked values subtracted by their NC are both assessed as screening responses.

The transformation appropriateness is evaluated by distribution of the responses on the dataset after exclusion of the outliers (so called blank population dataset). The blank population delineation is based on a mixed-effects model applying Tukey's outlier criterion on the conditional residuals and subject's Best Linear Unbiased Predictors (BLUP) for analytical and biological outlier identification, respectively. According to Tukey's outlier criterion, observations that fall below Q1 – 1.5*(Q3-Q1) or above Q3 + 1.5*(Q3-Q1) are considered as outliers, with Q1 and Q3 representing the 25th and 75th percentiles, respectively. Analytical outliers are removed before biological outliers, both in an iterative way.

The choice for the most appropriate blank population dataset (derived from either the difference of unspiked values and their NC or log-transformed ratios) is based on the normality assessment of the blank populations. If the blank screening population derived from the log-transformed ratios does not show significant evidence against normality by the Shapiro-Wilk test (p-value ≥ 0.05), SCPF setting is performed on this blank population. In case signifiant deviations from normality are seen on the log-transformed unspiked over NC ratios, the blank population delineated from unspiked values subtracted by their respective NC is evaluated. If no significant deviations from normality are seen here, this blank population is used for subsequent analysis. In case both blank populations return a p-value < 0.05 by the Shapiro-Wilk test, the blank population providing the smallest absolute value for the skewness coefficient is taken forward for SCPF setting.

Also the method of SCPF calculation is based on the normality properties of the obtained blank population. In case no evidence against normality is seen by the Shapiro-Wilk test (p-value ≥ 0.05), SCPF is determined by the parametric approach (mean + k (one-sided standard normal quantile) x SD (standard deviation)). This k value is based on the targetted false positive rate (FPR). If, however, evidence is provided for deviations from normality on the blank population dataset, but the absolute value of the skewness coefficient does not exceed 1, SCPF is obtained by the robust alternative method. Here, median is used instead of mean, and the SD is estimated by 1.4826 * median absolute deviation (MAD) to ensure robustness. In case the Shapiro-Wilk test shows significant deviations from normality (p-value < 0.05) and the absolute value of the skewness coefficient exceeds 1, both the robust alternative and the observed percentiles of the blank population (non-parametric method) are outputted for the determination of SCPF. In order to assure the selected FPR with a specified confidence level, the non-parametric SCPF are determined by their one-sided lower confidence limit as established by the smoothed empirical likelihood quantiles. For the 95th percentile a 90% one-sided confidence level, while for the 99th and 99.9th percentiles, the 80% one-sided confidence level is incorporated for the SCPF determination. In case log-transformed dataset is used, back-transformation is applied to obtain the SCPF.

In order to establish suitability of the SCPF, the NC results should represent the drug-naïve matrix sample results of the target population. Therefore, the NC values should drift in the same direction as the individual subject samples. This is assessed by the correlation of the plate's median (- if applicable – log-transformed) screening values versus plate's median (log-transformed) NC on the blank screening dataset. Also the correlation of the run's median on the whole dataset. Both Pearson's and Spearman's correlation coefficients should be positive in order to confirm suitability.

Although formal assessment of the analyst-specific differences is performed on sample's final ADA scoring, differences in means and variances are also assessed on the blank population as supportive information. Differences in means are assessed by the mixed effects model including {:Analyst} as fixed effect and {:Plate ID[:Analyst,:Run], :Run[:Analyst], :Subject ID} as random effects. Analyst-specific differences in variances on the blank population are assessed by a Levene's test. If analyst-specific differences in either means or

Report Output 2

Distribution plots & section summaries

4. Screening Cut-Point Factor

4.1. Assay Response (Log[Unspiked / Negative Control])

4.1.1. Distribution of Screening Response before Outlier Exclusion

Figure 5: Boxplots of log-transformed screening response values before outlier exclusion by analyst and run colored by plate

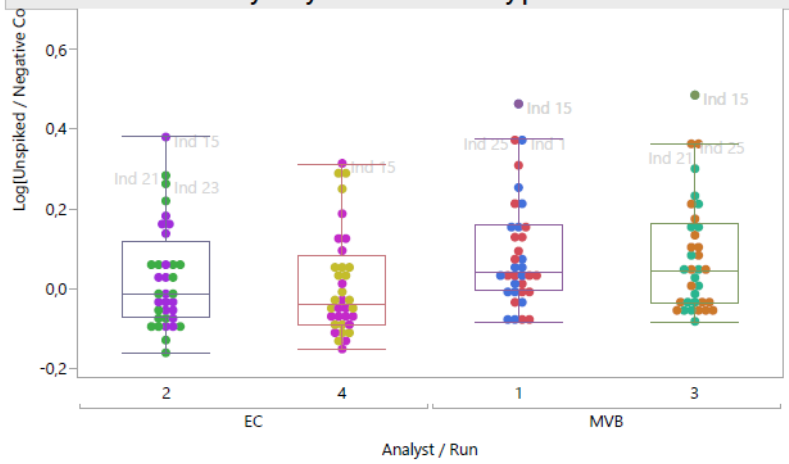
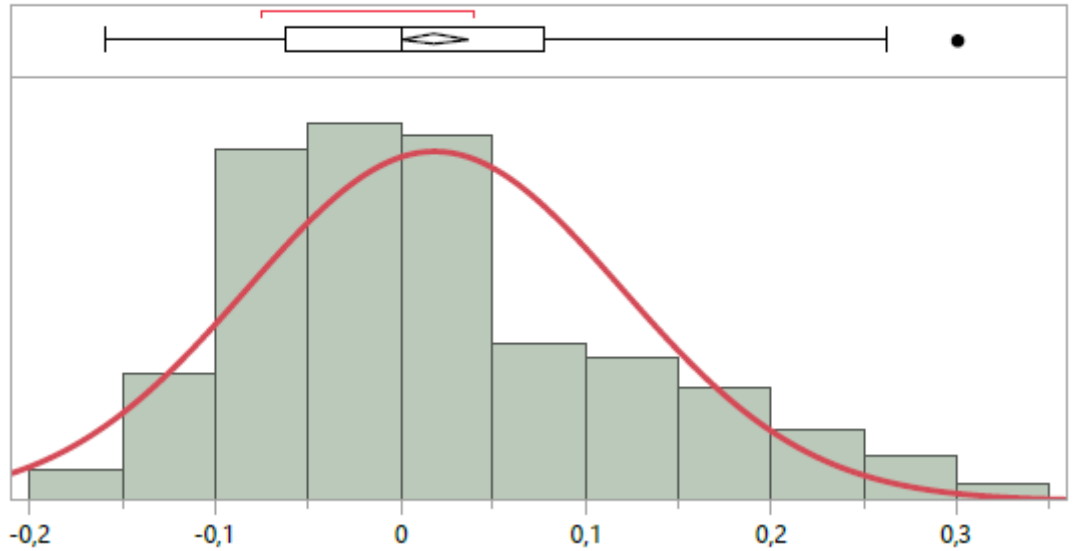


Figure 8: Distribution of log-transformed Unspiked / Negative Control assay response on dataset with analytical and biological outliers excluded



4.9. Conclusions on Screening Cut-Point Factor

Blank population has been delineated by iterative outlier removal approach based on the mixed effects model including Analyst as fixed effect and Plate ID (nested within Analyst and Run), Run (nested within Analyst) and Subject ID as random effects.

A **multiplicative screening cut-point factor of 1,167** has been obtained by the robust alternative approach allowing 5% FPR on the blank population.

For the cut-point factor for titration purposes allowing 0.1% FPR, a multiplicative titer cut-point factor of 1,337 has been established.

Suitability of the negative control for the screening cut-point factor could be confirmed.

Analyst-specific differences in means have been observed on the blank screening population.

Report Output 3

Overview scatterplots & final conclusions

- View of outliers, confirmatory and screening results
- Final conclusions includes summary of data, cut point factors (screening, confirmatory, titer and matrix interference) and summary of ADA scoring of samples.

Figure 4: Scatterplot of % inhibition versus screening response colored by Subject ID

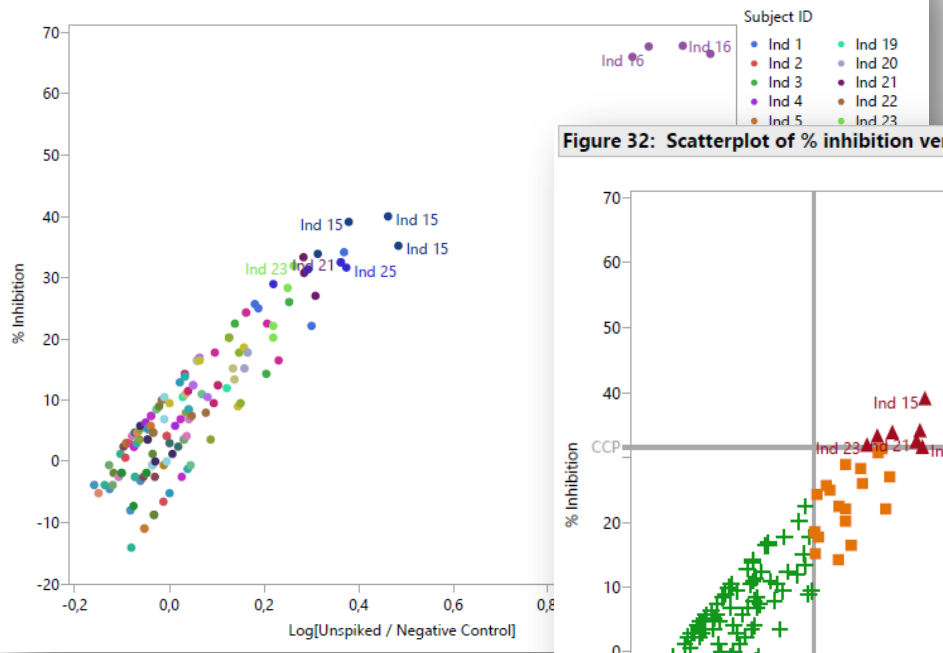
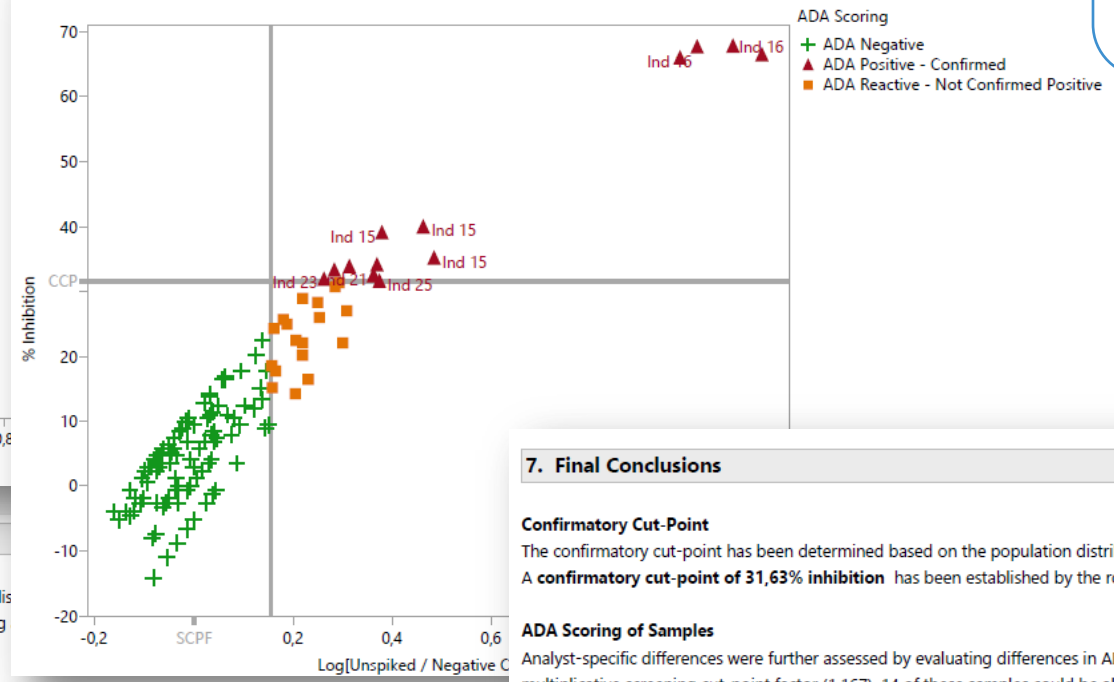


Figure 32: Scatterplot of % inhibition versus log[unspiked / negative control] values colored by ADA scoring



7. Final Conclusions

Dataset Descriptives

A total of 144 observations were included in the initial dataset with data of 36 dis
uploaded. As such, the analysis dataset is identical to the intial dataset, retaining

Screening Cut-Point Factor

Blank population has been delineated by iterative outlier removal approach based on the mixed effects model including Analyst as fixed effect and Pla
and Run), Run (nested within Analyst) and Subject ID as random effects.
The screening cut-point factor has been determined on the blank population derived from the Log[Unspiked / Negative Control] response values.
A **multiplicative screening cut-point factor of 1,167** has been obtained by the robust alternative approach allowing 5% FPR on the blank populatio
For the cut-point factor for titration purposes allowing 0.1% FPR, a multiplicative titer cut-point factor of 1,337 has been established.
Suitability of the negative control for the screening cut-point factor could be confirmed.

7. Final Conclusions

Confirmatory Cut-Point

The confirmatory cut-point has been determined based on the population distribution of the untransformed ratios of Spiked over Unspiked values.
A **confirmatory cut-point of 31,63% inhibition** has been established by the robust alternative approach allowing 1% false positives on the untransformed blank population.

ADA Scoring of Samples

Analyst-specific differences were further assessed by evaluating differences in ADA scoring. 33 samples in the dataset were scored as ADA reactive based on the established multiplicative screening cut-point factor (1,167). 14 of these samples could be also confirmed as ADA positive based on the confirmatory cut-point allowing 1% false positives on the derived blank population. No evidence is provided for analyst-specific differences in sample's ADA scoring.

8. Data Table(s)

The pdf file of the analysis data table _ADA_26Oct2021_Output Analysis Dataset.pdf has been outputted to folder \\frasdat113\ftpdata\BBB\BCB_global\JMP_CutPoint\Report\.

Conclusions and Learnings

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Valuable tool that takes dedicated resources to accomplish

- Value comes in automation, standardization & reduced operational complexity
- Allows state-of-the-art immunogenicity cut-point analysis, updates can be pushed to all teams simultaneously
- Standard preferred approach as default with flexible settings for many modalities & situations
- Quick and efficient – reduced effort of human task for analysis & reporting
- Includes acceptance criteria checks and diagnostic analysis evaluations
- Run in a validated environment and suitable for regulatory submissions

Presented at EBF Open Symposium Nov 24-26, 2021

Team Credits

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Carla Duymelinck – SQO
Liza Borms - SQO
Kamruzzan Biswas – VEX

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Valérie Martin – CSO BP
Eric Guillemare – BCB
Valerie Boutet – BCB

Germany

Joerg Roesser – BCB
Katharina Michalik-Gessler – BCB
Claudia Fink – BCB
Karin Benstein – TMPO
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Satish Behl – VEX

THANK YOU

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