# The New JMP 16 Limits of Detection in Design of Experiments and Data Analysis

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August 2021
US JMP Discovery Conference

# Limits of Detection

## Abstract

JMP 16 had new features that incorporate the limits of detection into Column Properties, are part of Design of Experiment platform, and are automatically applied during modeling with JMP Pro's Generalized Regression platform. I will demonstrate these new features, and discuss the impact of limits of detection on data analysis.

When we look for very small amounts of a substance, we are limited by the technology we are using. A substance could be present but we are unable to detect it. This is the lower limit of detection, and has a huge practical importance for detecting low levels of a substance in critical situations such as identifying the start of an infection, or correctly identifying small amounts of impurities. Ignoring the limits of detection in data analysis have serious impacts; basic statistics such as means and confidence intervals become biased and misleading. Modeling data with incorrect assumptions about the limits of detection lead to erroneous models. Limits of detection have implications in industrial applications such as pharmaceutical and chemical manufacturing, analytical chemistry, and diagnostic test design.

# Limits of Detection

## Agenda

- Background and Definition
- Statistical Considerations if Ignoring LoD
- Real World Evidence of Lack of Robustness in Rapidly Designed Products with LOD - COVID diagnostic tests
- Applications in JMP - Demo
  - Design of Experiments
  - Data Modeling

# How do we know if something is there?

# Limits of Detection

## Definition - Analytical Methods Differ

- FDA ICH Molecular Diagnostic Assays: LoD is the lowest concentration of a target that can be detected in ≥95% of repeat measurements. (The LoD is a measure of analytic sensitivity not clinical sensitivity.)

- International Union of Pure and Applied **Chemistry** (IUPAC) defines a method's detection limit as the smallest concentration or absolute amount of analyte that has a signal significantly larger than the signal from a suitable blank.

- Signal to Noise Ratios
- Calibration Curve Methodologies
- Etc, etc.

# Limits of Detection

## Definition - Analytic Methods Differ

- JMP:  The Detection Limits column property defines bounds beyond which the response cannot be measured.

- Can be upper and/or lower limits (right and/or left censored)

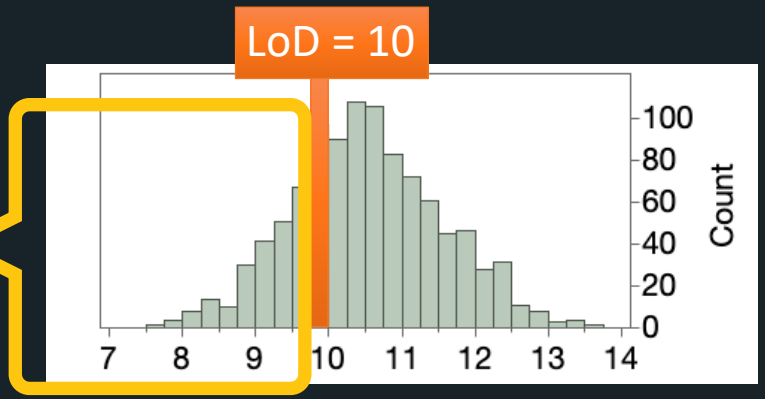
# Limits of Detection

## Statistical Considerations if Ignoring LoD

- True Population spans beyond LoD
- Below LoD can't measure, but it's ***not*** zero
- Correct statistical method is Censoring (Michael Crotty, Discovery 2017)

- If we ignore LoD = treat those measures as lower limit

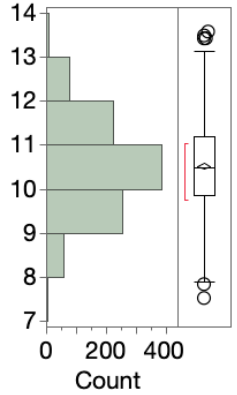
Treating this part of the population as at LoD



# Limits of Detection

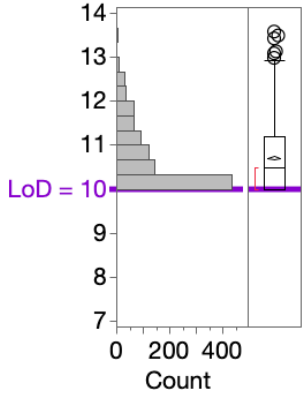
## Statistical Considerations if Ignoring LoD

**Impurity (true population)**



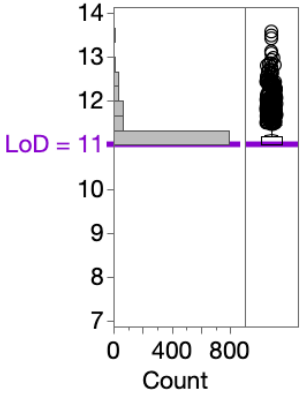

| Summary Statistics | |
|---|---|
| Mean | 10.5 |
| Std Dev | 1.0 |
| Std Err Mean | 0.0 |
| Upper 95% Mean | 10.6 |
| Lower 95% Mean | 10.5 |
| Variance | 1.0 |
| CV | 9.6 |
| Median | 10.5 |

**Impurity LoD=10**



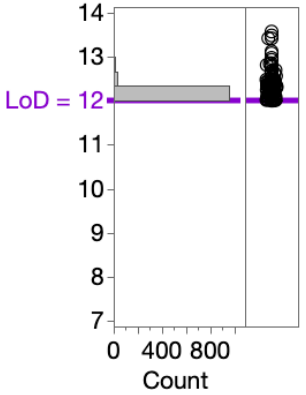

| Summary Statistics | |
|---|---|
| Mean | 10.7 |
| Std Dev | 0.8 |
| Std Err Mean | 0.0 |
| Upper 95% Mean | 10.8 |
| Lower 95% Mean | 10.7 |
| Variance | 0.6 |
| CV | 7.2 |
| Median | 10.5 |

**Impurity LoD=11**




| Summary Statistics | |
|---|---|
| Mean | 11.2 |
| Std Dev | 0.4 |
| Std Err Mean | 0.0 |
| Upper 95% Mean | 11.2 |
| Lower 95% Mean | 11.2 |
| Variance | 0.2 |
| CV | 3.9 |
| Median | 11.0 |

**Impurity LoD=12**




| Summary Statistics | |
|---|---|
| Mean | 12.0 |
| Std Dev | 0.2 |
| Std Err Mean | 0.0 |
| Upper 95% Mean | 12.0 |
| Lower 95% Mean | 12.0 |
| Variance | 0.0 |
| CV | 1.3 |
| Median | 12.0 |

# Limits of Detection

## Statistical Considerations if Ignoring LoD

| | Impurity (true population) | Impurity LoD=10 | Impurity LoD=10 formula | Impurity LoD=11 | Impurity LoD=11 formula | Impurity LoD=12 | Impurity LoD=12 formula |
|---|---|---|---|---|---|---|---|
| | 13.6 7.52 | 13.6 7.52 | 13.6 10 | 13.6 7.52 | 13.6 11 | 13.6 7.52 | 13.6 12 |
| 1 | 11.26 | 11.26 | 11.26 | 11.26 | 11.26 | 11 | 12.00 |
| 2 | 10.75 | 10.75 | 10.75 | 10.75 | 11.00 | 11 | 12.00 |
| 3 | 10.19 | 10.19 | 10.19 | 10.19 | 11.00 | 10 | 12.00 |
| 4 | 12.33 | 12.33 | 12.33 | 12.33 | 12.33 | 12 | 12.33 |
| 5 | 11.74 | 11.74 | 11.74 | 11.74 | 11.74 | 12 | 12.00 |
| 6 | 10.41 | 10.41 | 10.41 | 10.41 | 11.00 | 10 | 12.00 |
| 7 | 9.22 | 9.22 | 10.00 | 9.22 | 11.00 | 9 | 12.00 |
| 8 | 11.65 | 11.65 | 11.65 | 11.65 | 11.65 | 12 | 12.00 |
| 9 | 10.01 | 10.01 | 10.01 | 10.01 | 11.00 | 10 | 12.00 |
| 10 | 10.92 | 10.92 | 10.92 | 10.92 | 11.00 | 11 | 12.00 |
| 11 | 11.62 | 11.62 | 11.62 | 11.62 | 11.62 | 12 | 12.00 |
| 12 | 10.49 | 10.49 | 10.49 | 10.49 | 11.00 | 10 | 12.00 |
| 13 | 8.91 | 8.91 | 10.00 | 8.91 | 11.00 | 9 | 12.00 |
| 14 | 11.21 | 11.21 | 11.21 | 11.21 | 11.21 | 11 | 12.00 |
| 15 | 10.24 | 10.24 | 10.24 | 10.24 | 11.00 | 10 | 12.00 |

# Limits of Detection

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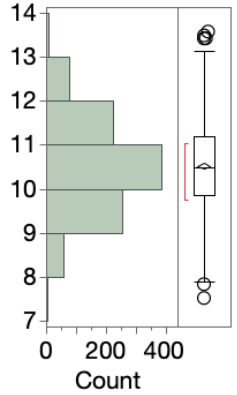


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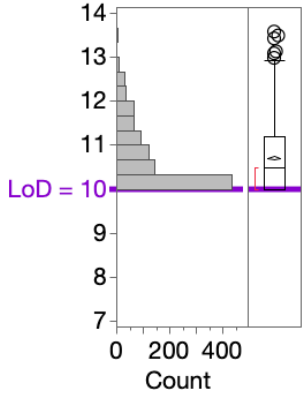


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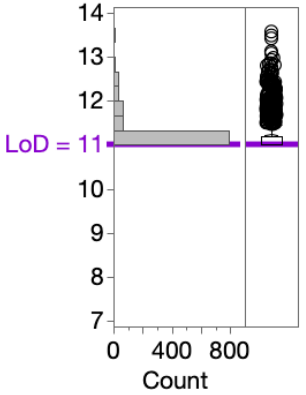


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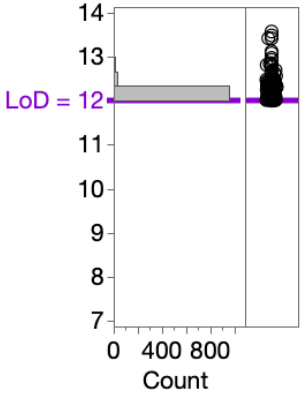


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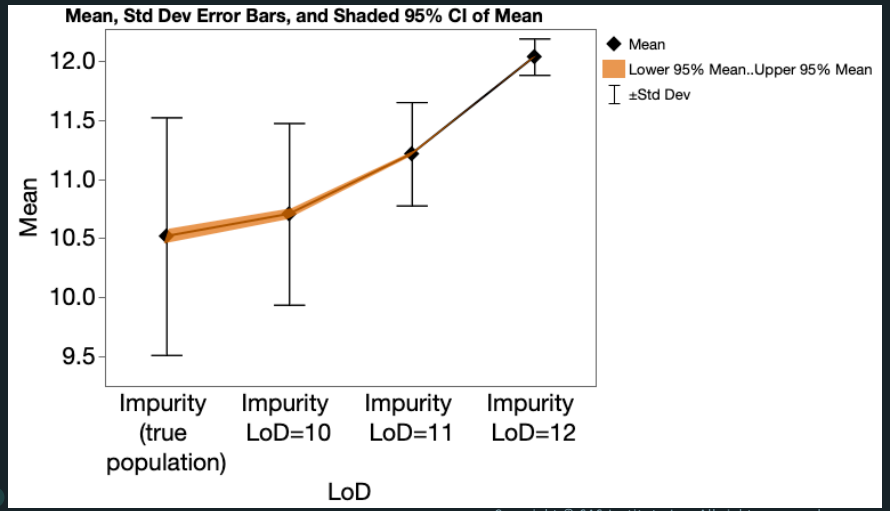
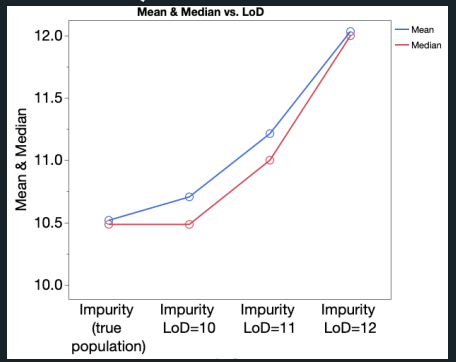
| Variance | 0.0 |
| CV | 1.3 |
| Median | 12.0 |

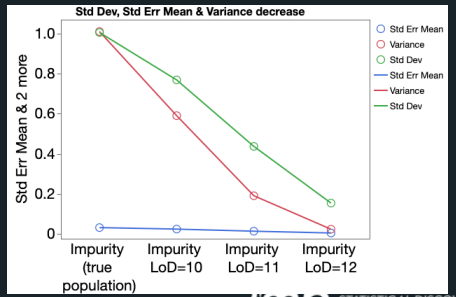
# Limits of Detection

## Statistical Considerations if Ignoring LoD

- Mean is biased away from true mean (lower LoD)
- Measures of variation decrease because truncating observations



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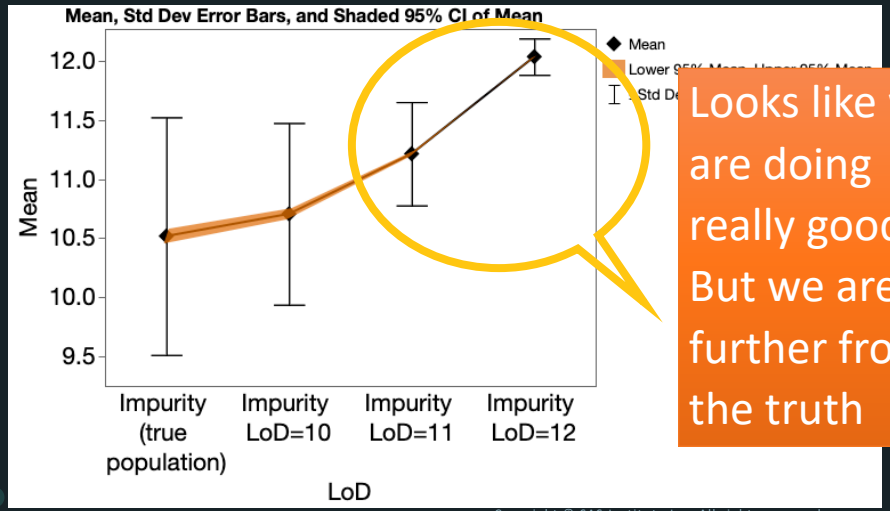
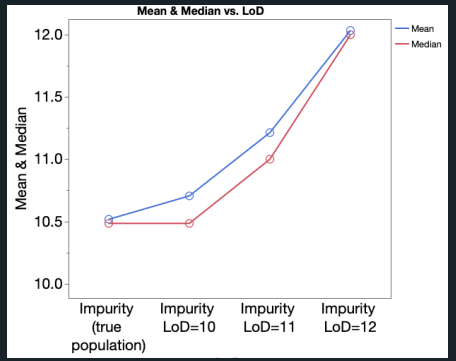
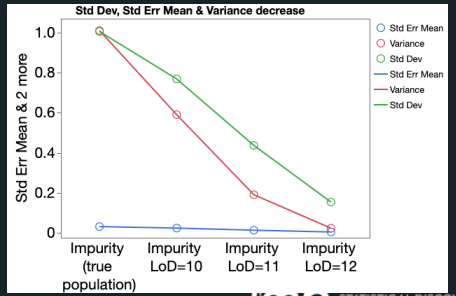



Looks like we are doing really good… But we are further from the truth



# Limits of Detection
## Design of Experiments

- If we do not use Limits of Detection (LoD) in Designed Experiments:
  - Not Find Critical Factors
  - Not Find the True Relationship Among Factors

# How Robust Were COVID-19 Diagnostic Tests Made?

# How Robust Were Diagnostic Tests Made?

## Early 2020: Limits of Detection as Diagnostic Tests for COVID-19 Active Infections

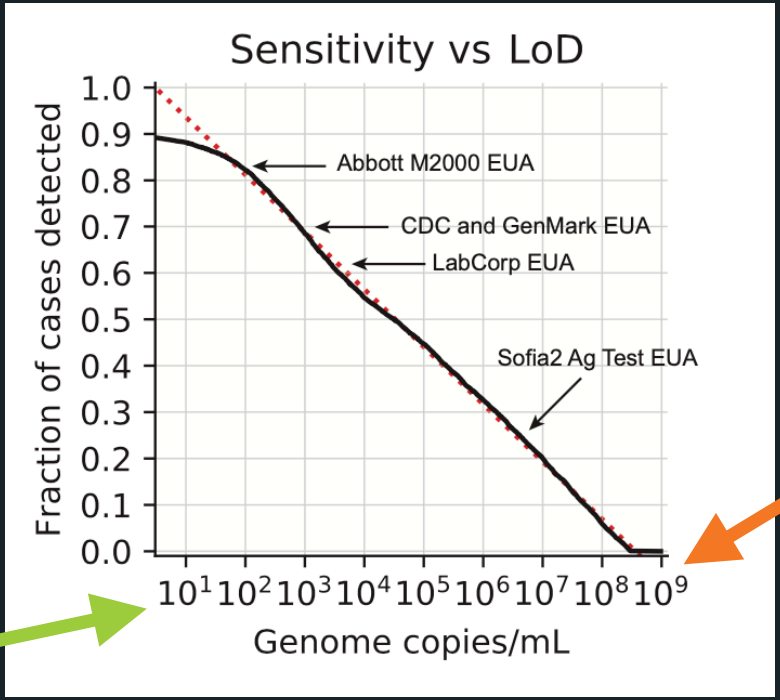
- FDA Emergency Use Authorization (EUA) given to >20 tests based on ***limits of detection (LoD)*** for active SARS-CoV-2 infections (COVID-19)
- RT-PCR test for Viral Load

- Initially, Manufacturers could use their own internal standard (because none existed)
- Later, FDA gave out a standard and re-assessed diagnostic tests

Compare the rank order of the tests based on LoD for the two standards

# LoD in Diagnostic Test Relate to Clinical Sensitivity

## Viral Load Detection and Fraction of Cases Detected




Smaller Viral Load Detected = Mild Infections

Large Viral Loads = Heavy Infection (Super Spreaders)

**Figure 2** LoD Matters • **Clinical Infectious diseases 2021: Arnaout et al.** https://doi.org/10.1093/cid/ciaa1382

# How Robust Were Diagnostic Tests Made?

## Early 2020: Limits of Detection as Diagnostic Tests for COVID-19 Active Infections

Compare the rank order of the tests based on LoD for the two standards

- April 2 = Manufacturers' own internal standard
- September 15 = FDA standard
- Rank Order of each manufacture's test using LoD for each time period
  - 1 = best

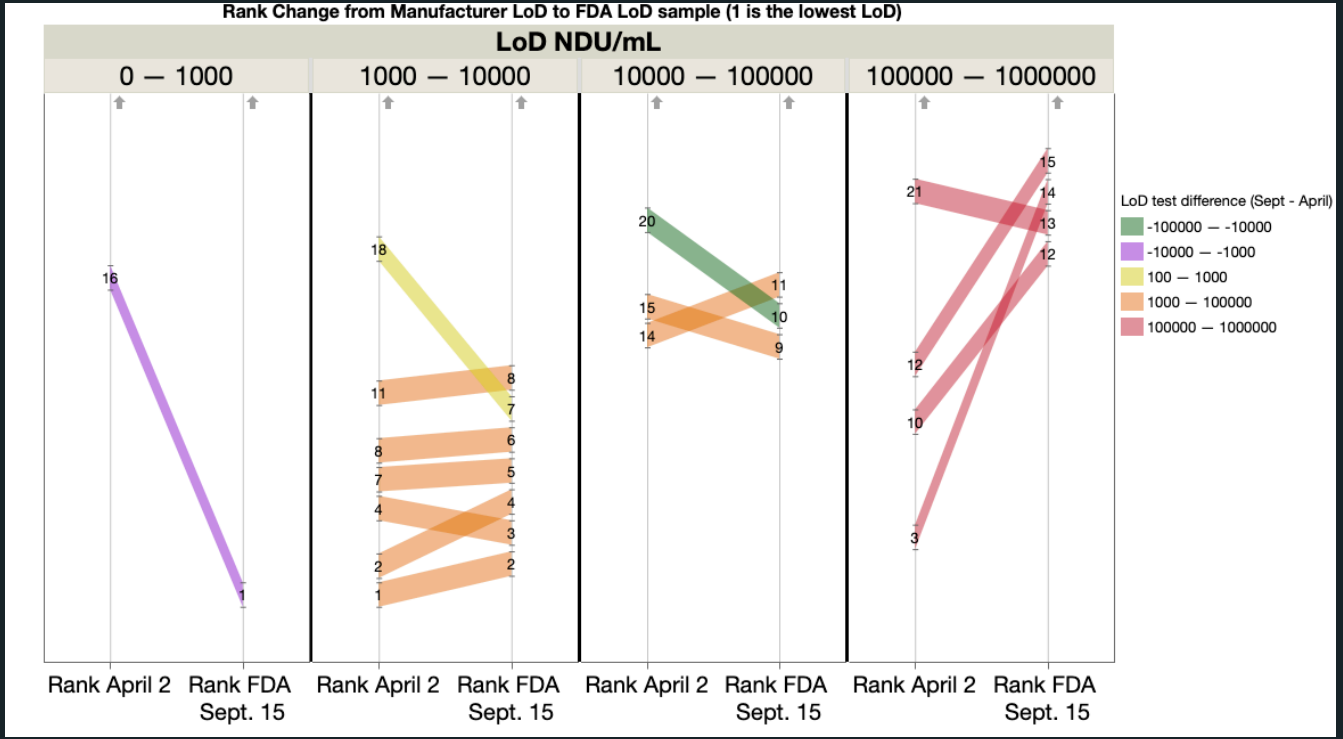
If diagnostic tests are robust = expect the same rank order and similar LoD

If diagnostic tests are not robust = expect change in rank order and varying LoD

LoD = Lowest amount of the virus that the test can detect at least 95% of the time

# How Robust Were Diagnostic Tests Made?

Change in standard from manufacture's to FDA standard resulted in differences in test rank for different manufacture's tests for limit of detection of viral load



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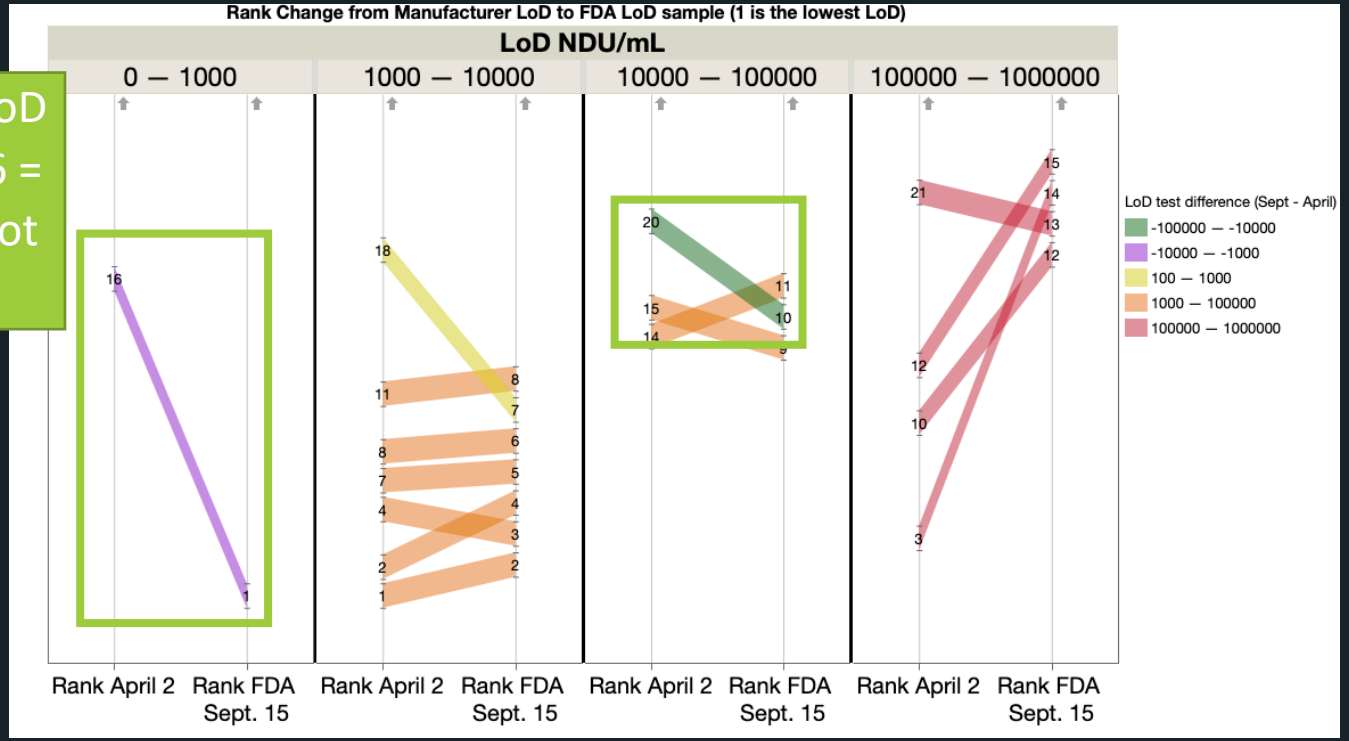
Change in standard from manufacture's to FDA standard resulted in differences in test rank for different manufacture's tests for limit of detection of viral load

Increase in LoD 10^5 to 10^6 = Some tests got much better



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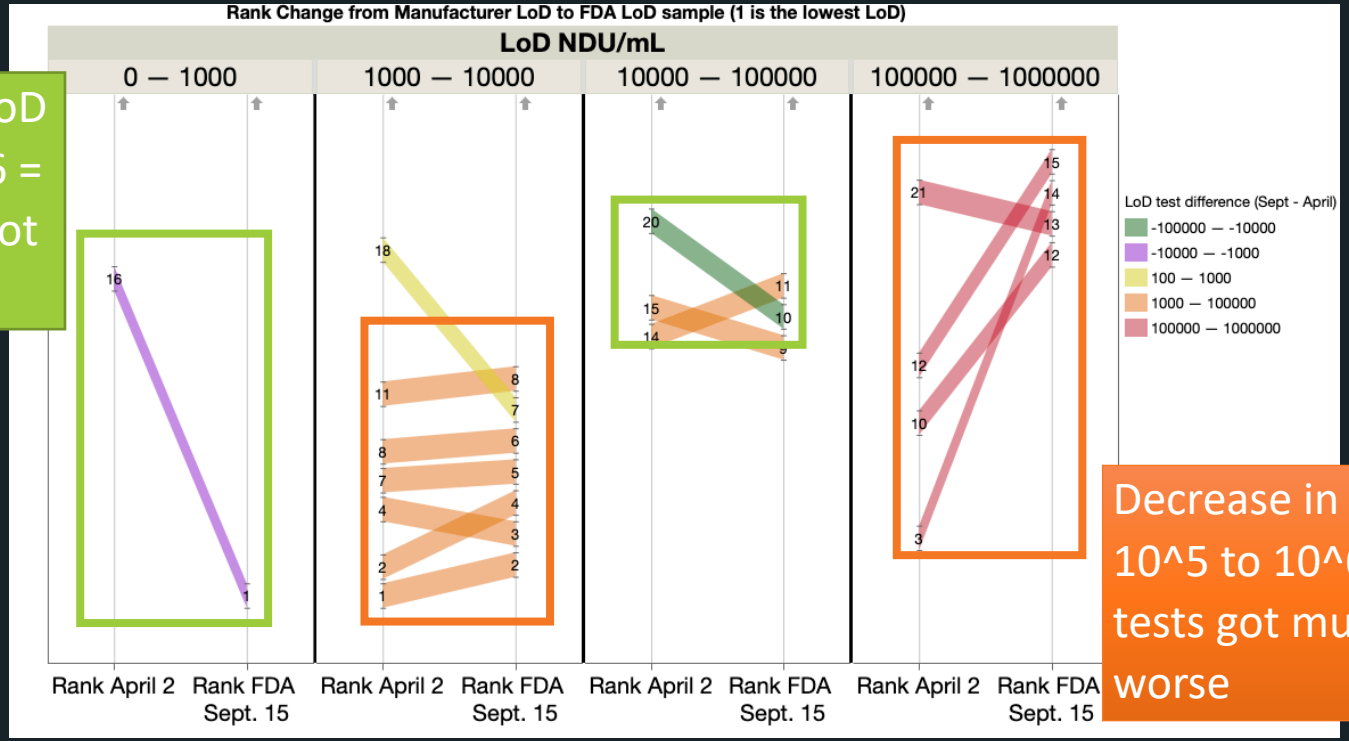



Decrease in LoD 10^5 to 10^6 = Some tests got much worse

# How Robust Were Diagnostic Tests Made?

Compare the rank order of the tests based on LoD for the two standards

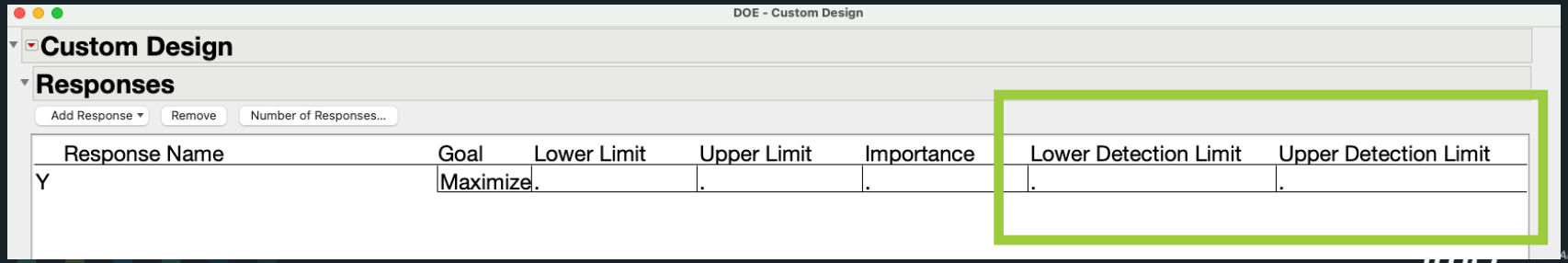
## Tests lack robustness

## How can we improve?

Use Limits of Detection in Design of Experiments for more robust designs

# Limits of Detection
## Design of Experiments

- Setting up Limits of Detection in constructing DoEs
  - Creates Column Property with LoD
  - Creates censored values when observation is outside of LoD in Generalized Regression Analysis



# JMP Demo

# Limits of Detection

## Design of Experiments and Analysis

- Goal: Optimize determination of a pesticide (Metacrate) from water samples

- Factors:
  - Dichloromethane (dispersive)
  - Methanol (solvent)
  - Water Sample Volume

- 32 run, I Optimal design

- Nine of the 32 observations were below the lower LOD of 1.0 %

# Limits of Detection

## Generalized Regression Analysis

- Analysis of data with LoD
- Compare to analysis not using LoD
  - 0, 1 (LOD = 1)
- Not using LoD results in missing factors, widely inaccurate results

# JMP Demo

# Limits of Detection

## Key Takeaways

- Limits of Detection are Important
- Easy to use LoD in JMP's DOE platforms
- Analysis with LoD give best model

Encourage people to re-analyze data where LoD was not used

# How Robust Were Diagnostic Tests Made?

## Early 2020: Limits of Detection as Diagnostic Tests for COVID-19 Active Infections

- A few details:

- LoD of Viral copies per ml = 22 diagnostic tests
- NDU/mL = NAAT (nucleic acid amplification test) Detectable Units/mL

LoD = Lowest amount of the virus that the test can detect at least 95% of the time

References


- SARS-CoV-2 Reference Panel Comparative Data https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-reference-panel-comparative-data
- Limits of detection for FDA-authorized COVID-19 diagnostics https://www.biocentury.com/article/630429/harmonizing-limits-of-detection-for-fda-authorized-covid-19-diagnostics

# Here to help

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