Calibration Verification Linearity in the Clinical Lab. Did I Pass or Fail?

2017 ASCLS New Jersey

AUDIT MicroControls

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Presentation Topics & Objectives

- Calibration Verification
  - Key Definitions
  - Why do I need to perform CV?
  - How do I perform CV?
  - When do perform CV?
  - Did my CV Pass Or Fail? *(* My organic chemistry final nightmare all over again)
  - Does a failed CV mean I need to retake the class? (Troubleshooting)

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Calibration Verification Definition

- Calibration tests the accuracy of the instrument.
  - Est. relationship between analyte content & instr. Measurement sig.

- Calibration Verification means testing materials of known concentration *in the same manner as patient specimens* to assure the test system is accurately measuring samples throughout the laboratory's reportable range of the instrument.

- Calibration verification tests the accuracy of the instrument *throughout the instrument's entire reportable range*

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Reportable Range

- Reportable Range is the span of test result values over which *the lab can establish or verify* the accuracy of the instrument or test system's measurement response
- It is the range of results for an analyte, from minimum to maximum, which *the instrument's test system can actually measure*
- The laboratory sets the Reportable Range
- After you run your calibration verification, this is what you will report

Analytical Measurement Range (AMR)

- **Defined by CAP as the range of numerical results a method can produce without any special specimen pre-treatment, such as dilution, that is not part of the usual analytical process (same as reportable range in CLIA terminology).**
- **AMR can be different from Reportable Range**
- ***AMR, unlike Reportable Range, comes from the manufacturer* and should show linearity over its entire AMR**

Why Perform Calib Verification

- **Per CLIA Sec. 493.1255 calibration verification procedures are required to substantiate the continued accuracy of the test system *throughout the laboratory's reportable range***
- **Non-waived moderately complex quantitative tests must have calibration verification performed!**
  - **Non-Waived**
    - **Moderately Complex**
    - **High Complexity**
  - **Waived**
  - **Quantitative**
  - **Semi-quantitative**

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How To Perform Calibration Verification

- Run at least three levels of material (CLIA requirement)
- Run a low, middle and high level as if running patient samples
- CLIA does not specify the number of replicates (recommend at least two replicates as GLP to pick up potential outliers)
- Record data
- Document results and determine if the results meet the laboratory's acceptable criteria

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Acceptable Cal Ver Material

- Materials of known concentration whose known upper and lower values are near the upper and lower values of the instrument. Must also include a mid-range and have enough volume of sample to complete the run
  - Calibration Material
  - Proficiency testing samples with known results
  - Controls with known values
  - Previously tested patient samples
  - Commercially available material

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When To Run Calib Verification

Per CLIA 493.1255(b)(3)

- **Labs must perform every six months (or more frequently if specified in the test system's instructions) or if:**
  - **There is a complete change of reagents for a procedure – unless the lab can show that the new lot of reagents does not affect the range of the patient's test results and that daily quality control results are not adversely affected by changing reagent lots**
  - **CLIA's probe for 493.1255(b) 'If a laboratory does not perform CV after a complete change of reagents, what data does the lab have to document that changing the reagent lot numbers does not affect the reportable range of patient test results, and does not adversely affect control results?'**
  - **Use your routine QC data (5 controls before and 5 after the reagent change) to show a shift has not occurred**

When To Run Calib Verification

CLIA 493.1255(b)(3)cont.

- **Major preventative maintenance or replacement of critical instrument components which may affect patient test results**
- **Control materials reflect an unusual trend or shift, or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem**
  - **Did analyzer get moved?**
  - **Different technician on the analyzer?**
  - **Change in analyzer protocol?**
- **The laboratory's established schedule for verifying the RR for patient test results requires more frequent calibration verification**

Why Calib Verification Matters

- Performing QC does not generally challenge the upper and lower limits of the instrument
- It is possible to have a passing QC and a failing CV
- The instrument's test system may have a limited number of calibrators
- Test method calibrators may not span the entire reportable range of the instrument, even with three calibrators
- Can detect Hook Dose Effect
- Patient presents significantly high or low
- Want to give results to physician with confidence

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So, Did I Pass?
What Are Acceptable Results?

- Plot data on linear graph
  - Individualized Custom Computer program
  - Commercial third party/manufacturer program
- Values obtained from running the test samples as patient samples are compared to the known values (peer data) of the material.
- If the values are acceptable, calibration of the analyte is verified

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So, Did I Pass?
What Are Acceptable Results?

- Each laboratory is responsible for establishing acceptable parameters
- Some labs use CLIA ‘88 Proficiency Testing Limits
- General Rule of Thumb used by some inspectors and laboratory managers:
  - R Coefficient (R^2): 0.98 – 1.00
    - Tells data is linear
  - Slope: 0.90 – 1.10 (+/- 10%)
    - Tells how well you match up to the expected data

Sample Worksheet for Calibration Verification

Worksheet and Documentation Form for Calibration Verification

Analyte _____ Date _____

Instrument _____ Serial Number _____

Reagent/Strip/Cassette Lot# _____ Expires: _____

Materials used _____

Source of Acceptable Limits _____

	Low Level	Mid Level	High Level
Lot Number			
Expiration Date			
Expected Value			
Acceptable Limits			

Calibration Verification Results

	Low Level	Mid Level	High Level
Results Obtained Repetition 1			
Optional: Repetition 2			
Repetition 3			
Mean			
Results Acceptable?			
Comments and/or Corrective Actions			

Performed by _____

Reviewed by _____ Date _____

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So, Did I Pass? #1

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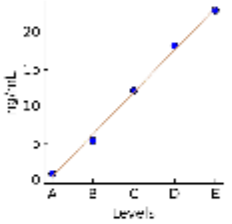
Linearity Report

Company Name: General Hospital
Lab Name: Chemistry Lab
Analyzer Model: AnyChem Analyzer
Analyzer Name: AnyChem SN #12345
Date Of Run: Dec 31, 2014
Technician: John Doe

Analyte: Folate
Analyte Units: ng/mL
Reagent: AnyChem Folate Reagent
Product: KIT4U-5 - Linearity FD Instrumentation
Lot Number: 5678
Lot Expiration Date: Sep 10, 2015

USER DATA SUMMARY



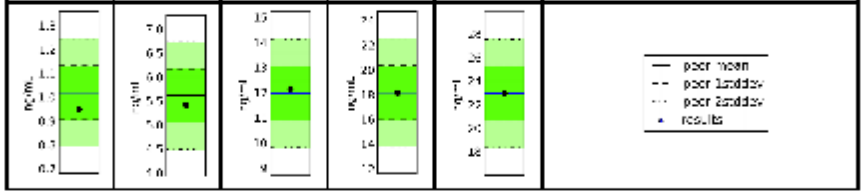
Mean	Y-Intercept	R-Squared
1.032	1.052	0.998

Level	Theoretical*	User Mean	Replicates
A	0.963	0.993	0.98, 0.98, 0.95
B	6.479	6.49	4.50, 6.62, 6.97
C	11.993	12.3	12.34, 12.48, 11.88
D	17.519	18.29	18.19, 18.67, 18.49
E	23.033	23.533	23.37, 22.33, 23.40

*Theoretical points determined from a model line generated between lowest and highest recovered values

USER VS. PEER COMPARISON
(100 Peers)

	Level A	Level B	Level C	Level D	Level E	Key
N	200	200	200	200	200	Number of peer data points
Peer Mean	1.023	6.623	12.012	18.230	23.100	Mean of the peer data points
User Mean	0.993	6.49	12.3	18.29	23.533	Mean of user replicates
+/- Diff	-0.07	-0.133	0.188	0	-0.07	Difference between user and peer means
% Diff	-6.8	-6.4	1.6	0.0	-0.3	% Difference between user and peer means
Peer Median	0.113	6.662	1.391	2.188	2.310	Peer standard deviation
Peer CV (%)	11.0	15.0	9.0	12.0	10.0	Peer median absolution



Approved By: _____ Date: _____

Each lot of product is manufactured such that a linear relationship exists among levels. Actual results obtained may vary depending on instrumentation, methodology, and assay temperature. Results may also be dependent on the accuracy of the instrument/reagent system calibration. The degree of acceptable non-linearity is an individual judgment based on methodology, clinical significance, and medical decision levels of the test analytes. This report is intended solely as a guide and are not to be interpreted as an absolute for a parallel judgment. AUDIT MicroControls, Inc. assumes no responsibility for the reading and interpretation of the above data.

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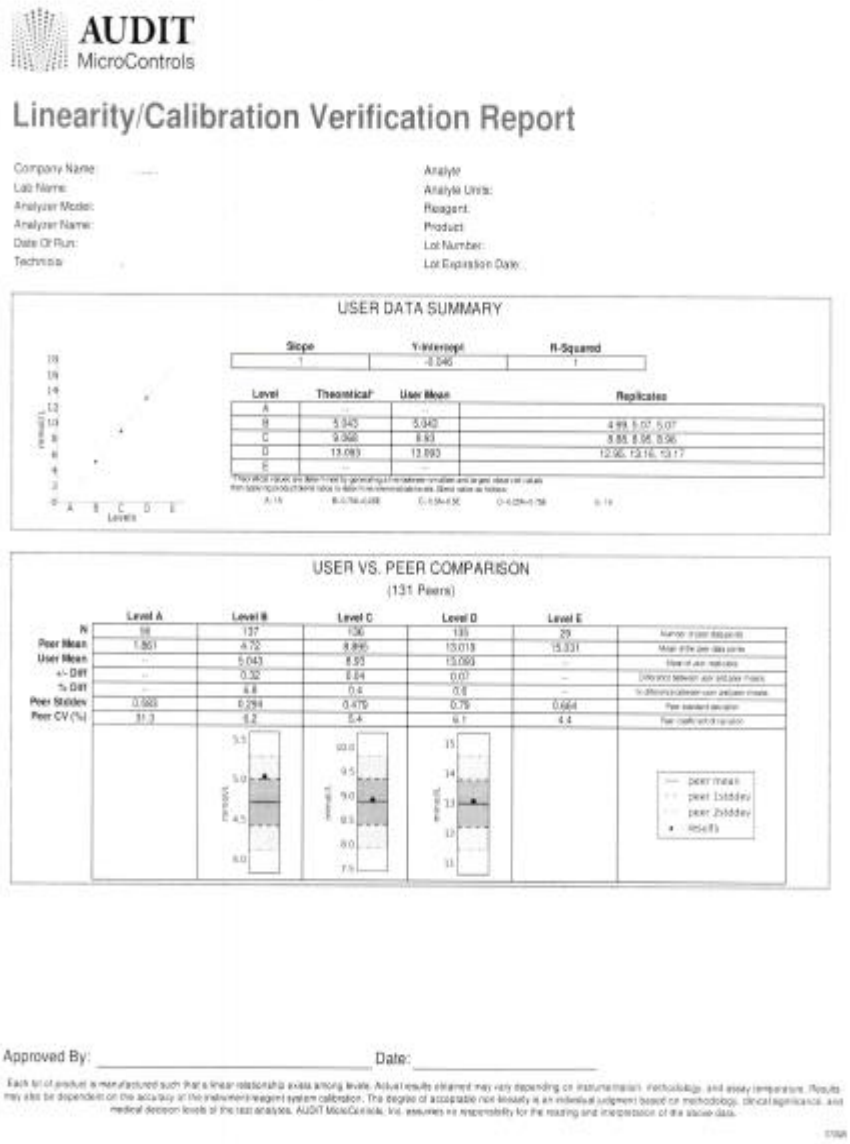
So Did I Pass? #2



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Linearity/Calibration Verification Report

Company Name:
Lab Name:
Analyzer Model:
Analyzer Name:
Date Of Run:
Technician:

Analyte:
Analyte Units:
Reagent:
Product:
Lot Number:
Lot Expiration Date:

USER DATA SUMMARY

Slope	Y-Intercept	R-Squared
1	-0.046	1

Level	Theoretical*	User Mean	Replicates
A	---	---	
B	5.043	5.043	4.99, 5.07, 5.07
C	9.068	8.93	8.86, 8.96, 8.96
D	13.093	13.093	12.96, 13.16, 13.17
E			

USER VS. PEER COMPARISON

(131 Peers)

	Level A	Level B	Level C	Level D	Level E	
N	18	137	136	135	29	Number of User Replicates
Peer Mean	1.801	4.72	8.896	13.013	15.031	Mean of the peer data points
User Mean	---	5.043	8.93	13.093	---	Mean of user replicates
+/- Diff	---	0.32	0.04	0.07	---	Difference between user and peer means
% Diff	---	6.8	0.4	0.5	---	% difference between user and peer means
Peer Stddev	0.693	0.294	0.479	0.79	0.664	Peer standard deviation
Peer CV (%)	31.3	6.2	5.4	6.1	4.4	Peer coefficient of variation

Approved By: _____ Date: _____

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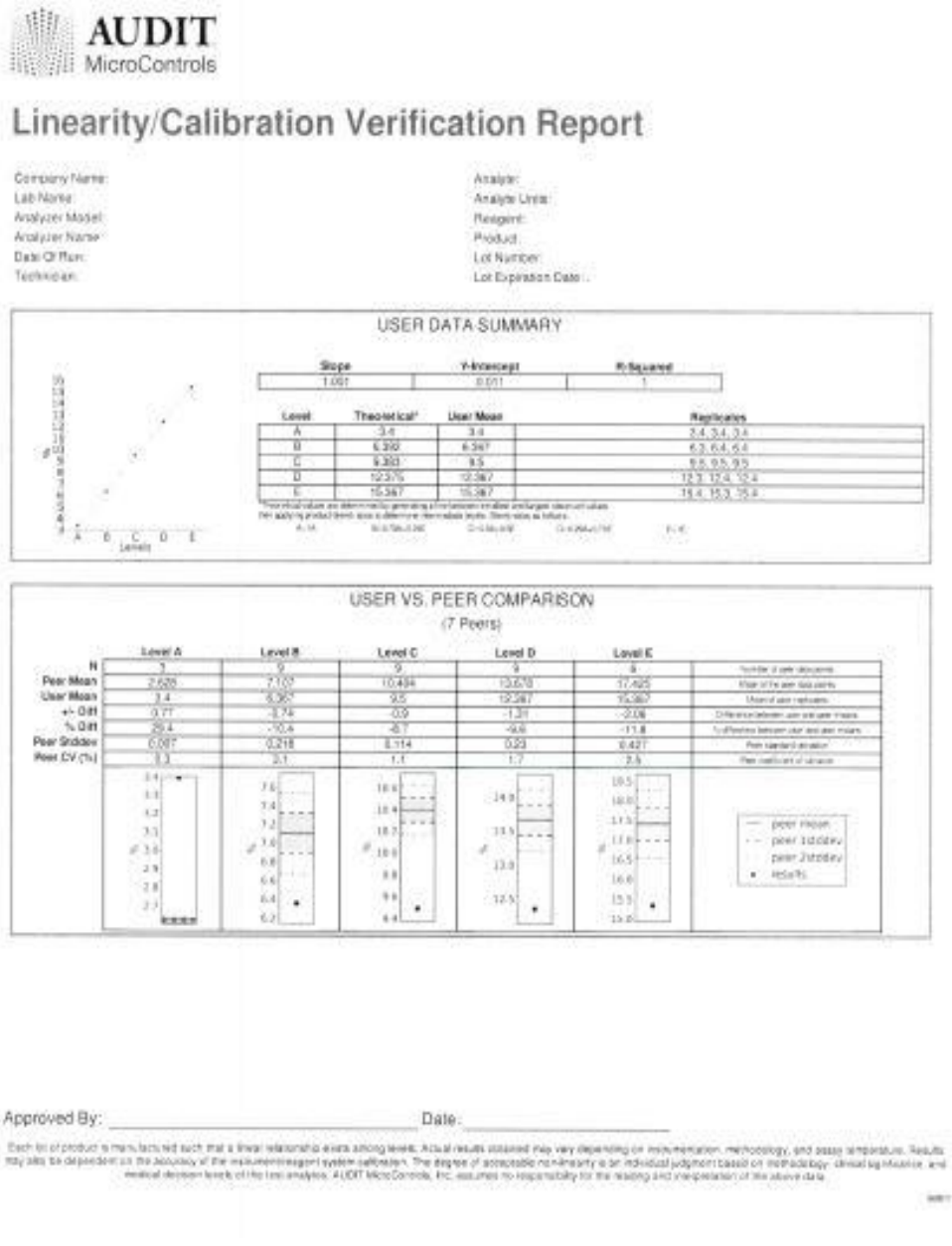
So, Did I Pass? #3



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Linearity/Calibration Verification Report

Company Name:
Lab Name:
Analyzer Model:
Analyzer Name:
Date Of Run:
Technician:

Analyte:
Analyte Units:
Reagent:
Product:
Lot Number:
Lot Expiration Date:

USER DATA SUMMARY

Slope	Y-Intercept	R-Squared
1.001	0.011	1

Level	Theoretical*	User Mean	Replicates
A	3.4	3.4	3.4, 3.4, 3.4
B	6.360	6.367	6.3, 6.4, 6.4
C	9.353	9.5	9.6, 9.5, 9.5
D	12.375	12.367	12.3, 12.4, 12.4
E	15.367	15.367	15.4, 15.3, 15.4

*Theoretical values are determined by generating a line between endlevel and largest value of values. Then applying product blend, assay is determined. Intermediate levels (blank, value, as follows:

USER VS. PEER COMPARISON

(7 Peers)

	Level A	Level B	Level C	Level D	Level E
N	7	9	9	9	9
Peer Mean	2.628	7.102	10.494	13.578	17.495
User Mean	3.4	6.367	9.5	12.367	15.367
+/- Diff	0.77	-0.74	-0.9	-1.21	-2.08
% Diff	29.4	-10.4	-8.7	-9.8	-11.8
Peer StdDev	0.087	0.218	0.114	0.23	0.427
Peer CV (%)	3.3	3.1	1.1	1.7	2.5

peer mean
peer 1stddev
peer 2stddev
results

Approved By: _____ Date: _____

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So Did I Pass? #4

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Linearity/Calibration Verification Report

Company Name:
Lab Name: Primary
Analyzer Model:
Analyzer Name:
Date Of Run:
Technician:

Analyte:
Analyte Units:
Reagent:
Product:
Lot Number:
Lot Expiration Date:

USER DATA SUMMARY

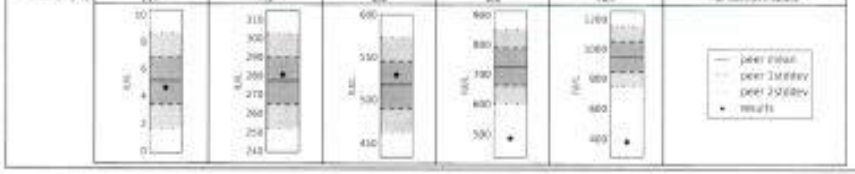
Slope	Y-Intercept	R-Squared
1.019	139.314	0.534

Level	Theoretical*	User Mean	Replicates
A	4.667	4.667	5, 5, 4
B	90.917	251	285, 278, 280
C	195.167	530	531, 524, 535
D	290.417	489	743, 319, 405
E	385.667	385.667	359, 414, 384

USER VS. PEER COMPARISON

(305 Peers)

	Level A	Level B	Level C	Level D	Level E	
N	489	498	487	332	54	Number of labs reporting
Peer Mean	5.183	277.798	518.008	727.309	955.243	Mean of the peer reported
User Mean	4.667	251	530	489	385.667	Mean of user replicates
+/- Diff	-0.52	-3.2	11.99	-238.31	-569.58	Difference between user and peer means
% Diff	-10	-1.2	2.3	-32.8	-59.6	% difference between user and peer means
Peer Stddev	1.748	12.584	27.682	82.564	101.774	Peer standard deviation
Peer CV (%)	33.7	4.5	5.3	11.3	10.7	Peer coefficient of variation



Approved By: _____ Date: _____

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What Does It All Mean?

- A successful calibration verification confirms that the test system is providing accurate results for the analyte throughout the reportable range of the test
- You need to challenge the entire range of what the manufacturer claims, to the best of your ability
- Clinical significance, the patient population
- Peer Data helps!

What If
Calibration Verification Fails

- If CV fails, it means the instrument calibration for this analyte may no longer be valid and corrective action is needed
  - Review QC Results
    - Patterns/values + or – mean?
    - Shifts/trends over time?
    - Are Accuracy & precision acceptable?
  - Check CV Material for OVS & CLS
  - Check Reagents
    - Reagent change?
    - New Lot#?
    - Manufacturer change?
    - New formulation of reagent ?
  - Review Instrument Maintenance Log
    - Missing data/doc's; Problems? Changes?

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Additional Troubleshooting

- Environmental changes
  - Instrument moved?
- Service Records
  - Serviced of late?
  - Software/hardware upgrades or changes?
- Instrument Operation
  - New instrument operators?
  - All operators following est. protocol?
- Check Comparative Method
  - Another lab nearby to compare results?
- Rerun Cal Ver
- If instrument is factory calibrated, call mfg.

Troubleshooting Checklist

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Troubleshooting Checklist for Calibration Verification

Analyte _____ Date _____

Instrument _____ Serial Number _____

1. Check your quality control (QC) results for the analyte	
• Are there any patterns seen in the control results?	
▪ Are all values below the mean?	Yes / No
▪ Are all values above the mean?	Yes / No
• Are there any noticeable shifts or trends over time?	Yes / No
• Are accuracy and precision acceptable?	Yes / No
Comments:	
2. Check your calibration verification material	
• Are the materials used appropriate and in-date?	Yes / No
• Have you properly determined the acceptable limits for the calibration verification material?	Yes / No
Comments:	
3. Check your reagents	
• Have there been any reagent changes?	Yes / No
• Is there a new lot number of reagent?	Yes / No
• Has there been a change in manufacturer?	Yes / No
• Has there been a new formulation (check the package insert) of current reagent?	Yes / No
• Are the reagents in date?	Yes / No
Comments:	
4. Check instrument maintenance	
• Review the daily, weekly, monthly, quarterly, etc. logs. Is there any missing maintenance, problems, or changes?	Yes / No
Comments:	
5. Check the environment	
• Has the instrument been moved recently?	Yes / No
• Have there been any changes to the environment or surroundings of the instrument?	Yes / No
Comments:	
6. Check the service record	
• Has the instrument been serviced recently?	Yes / No
• Has there been any software or hardware upgrades or changes?	Yes / No
Comments:	
7. Check instrument operation	
• Are there new instrument operators?	Yes / No
• Are all operators following established procedures for instrument operation?	Yes / No
• Has there been any recent modification to the technique used to run the test?	Yes / No
Comments:	
8. Check a comparative method	
• Is there another laboratory nearby that can run your calibration verification material so you can compare the results?	Yes / No
Comments:	
9. Will recalibration be performed for the analyte?	Yes / No
Comments:	

Thank You!!

If you have any questions or comments please feel free to contact me:

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