Validation of
Automated Inhibition Zone Reading using PD-100 compared to Manual EUCAST Reading

2026-03-30

Foreword

Antibiotic susceptibility testing using disk diffusion requires a high level of accuracy and standardization. More efficient tools are needed to meet future demands. The volume of samples is increasing, while access to trained personnel is limited.

The new technology in the PD-100 helps us address this challenge by providing reproducible results with full traceability while also improving ergonomics. The PD-100 represents a modern solution for a fundamental method in clinical microbiology.

Ingegerd Sjögren, Microbiologist, Ph.D.

Mikrobiologi Halland – Hallands Sjukhus Halmstad, Sverige

2026-03-09

Executive Summary

This validation study conducted at Hallands Sjukhus evaluated the analytical performance and operational efficiency of the PD-100 automated inhibition zone reader compared with manual EUCAST-compliant disk diffusion reading under routine laboratory conditions.

The validation confirms that PD-100 delivers analytical performance equivalent to manual reference reading when used with standard operator verification.

Key findings include:

- **98%** overall agreement with manual reference reading after operator verification
- **100%** disk identification accuracy
- **100%** categorical agreement (S/I/R interpretation)
- No very major errors (VME) or major errors (ME) observed
- Full compliance with EUCAST disk diffusion methodology

Automated-only measurements demonstrated high performance for routine MH plates and, following operator verification, achieved consistently high agreement across all plate types, including complex MHF plates.

In addition to analytical equivalence, PD-100 demonstrated measurable workflow improvement. Based on median processing times and the laboratory's normal plate distribution, the system reduced processing time by up to 52%, corresponding to approximately 31 minutes saved per 100 plates per day.

Beyond time efficiency, PD-100 enhances laboratory quality by enabling standardized measurements, eliminating manual data transcription, providing full image-based traceability, and strengthening audit readiness.

Overall, the validation supports safe implementation of PD-100 in routine EUCAST disk diffusion workflows, delivering reliable analytical performance combined with improved operational efficiency and documentation quality.

Content

Validation Report

Validation of Automated Inhibition Zone Reading using PD-100 compared to Manual EUCAST Reading

1 Purpose

The purpose of this study was to validate the performance of the PD-100 automated zone reader by comparing automated inhibition zone measurements with manual readings performed by experienced laboratory personnel, in accordance with EUCAST disk diffusion methodology.

Focus areas:

Performance evaluation of the PD-100 automated inhibition zone reader was conducted in accordance with general performance and safety principles applicable to in vitro diagnostic medical devices, with focus on:

- Measurement accuracy
- Agreement with manual reference method
- Repeatability
- Traceability and documentation
- Workflow efficiency

2 Background

Disk diffusion remains a widely used antimicrobial susceptibility testing method and requires strict adherence to standardized methodology to ensure reliable and reproducible results.

The manual EUCAST disk diffusion reading method was used as the reference standard, see Ref. [1], EUCAST requires all inhibition zones to be measured visually at complete growth inhibition and recorded to the nearest millimetre. Automated zone readers may be used provided they are calibrated against manual reading.

3 Materials and Methods

The study was carried out at Hallands Sjukhus in Halmstad, Sweden together with the Chief Medical Scientist in Mikrobiologi.

The agar plates were from daily patient samples with a focus on reading the plates manually and at the same time using the PD-100 to measure the zones. The plates selected were randomly chosen, with a particular focus on MHF plates, since these are the most challenging to interpret for the PD-100.

The functionality of the PD-100:

- The PD-100 takes a photo of the agar plate with both high and low exposure
- The same algorithm SA Yellow was used for all plates
- After advanced analysis PD-100 presents the suggestions for the antibiotic disks and the measured inhibitions zones
- The technician easily corrects (if needed) and approves the results, see *Figure **1*** for the PD-100 software program
- PD-100 saves photos and measuring results in a format, that can be transferred to the laboratory's own LIMS system

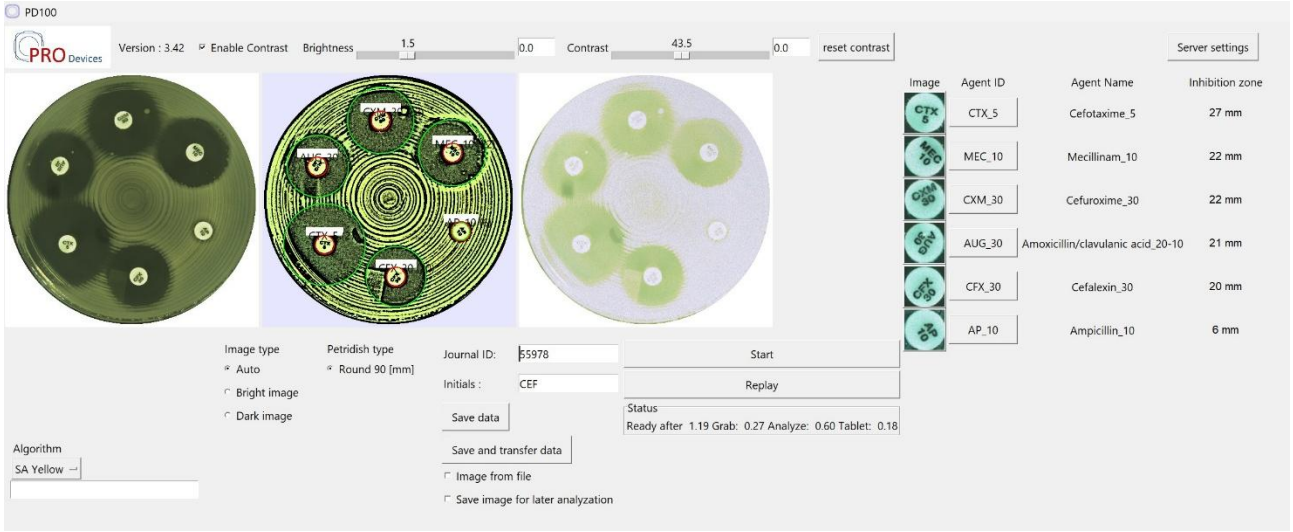

Figure 1 – PD-100 with results

Manual Method:

- Performed by two experienced laboratory staff
- One operator measured the inhibition zones
- One operator entered data into the Laboratory Information Management System (LIMS)
- The time was noted from the plate was taken, until the measurements were done and data typed

Automated Method (PD-100):

- Automatic disk detection
- Automatic zone measurement
- Automatic transfer to LIMS
- Image storage for traceability and re-evaluation
- Operator review and adjustment when required
- The time was noted from the plate was taken, placed in the PD-100 and until it was taken out again, if adjustment was needed the time for this was noted separately

3.1 Study Design – 25 different agar plates

In the study a total of 25 different agar plates were analysed, both Mueller-Hinton (MH) and Mueller-Hinton + Horse Blood (MHF) to investigate the accuracy of the PD-100.

Distribution:

- MH plates: 16 plates – with a total of 69 zones
- MHF plates: 9 plates – with a total of 40 zones

Generally, the MH plates are much easier to measure than the MHF plates, both by the manual process with a calliper and by the PD-100.

3.2 Study Design – 25 measurements of the same agar plate

To test the repeatability of the PD-100, one (1) MH plate was measured manually once and 25 times with the PD-100. The plate was turned in the PD-100 between each measurement, see *Figure **2***.

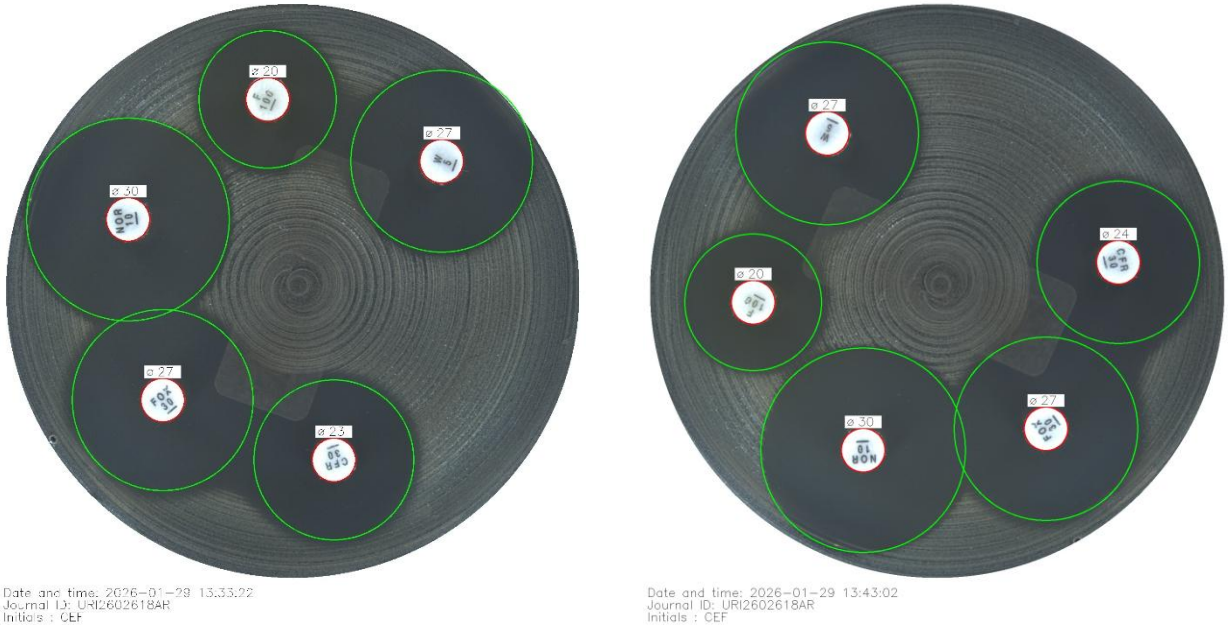

Figure 2 – Same plate measured twice

4 Acceptance Criteria

The comparison of automated, camera-based inhibition zone measurements against manual, "golden standard" calliper measurements typically require high concordance. The following criteria have been found in other literature, see Ref. [3] – Ref. [5]. The criteria for Categorial Agreement can be found in Ref. [7].

Metric	Acceptance Criteria
Barcode/Label Scanning	> 99%
Disk Recognition Accuracy – The software should identify the antibiotic disk and its concentration	> 99%
Categorical Agreement (CA) – agreement in S, I, R interpretation[1]	≥ 90–95%
Zone Measurement Agreement[2]	≥ 90% within ±2 mm
Zone Measurement Agreement for same plate – Repeatability	≥ 95% within ±1 mm
Very Major Error (VME) – False Susceptible, a resistant isolate incorrectly reported as susceptible (S)	≤ 1,5%
Major Error (ME) – False Resistant, a susceptible isolate incorrectly reported as resistant (R)	≤ 3%

Measurement Deviation Classification:

- GREEN: ≤ ±2 mm
- YELLOW: ±2–3 mm
- RED: > ±3 mm

[1] Agreement in S/I/R made according to EUCAST, see Ref. [2]

[2] Zone diameter differences between automated and manual readings should be within ±1–3 mm, the articles from the references all use ±3 mm, we have aimed at ±2 mm

5 Results

All the plates were visually evaluated concerning the following Characteristics, and all were very similar:

Characteristics	MH Plates	MHF Plates
Inoculum density (Even/Uneven)	Even	
Inoculation method (Circular/Striped)	Circular	
Growth (Distinct/Poor)	Distinct	Poor
Contrast (High/Low)	High	Low
Label type	White with Black glue side	
Inhibition zone affected by label edge	None	
Marker ink on plate underside	No	
Number of wrong inhibition zones before adjustment	4 out of 69	27 out of 40
Number of overlapping inhibition zones	18 out of 69	14 out of 40
Number of zones with overlap and incorrect zone	3 out of 69	9 out of 40
Number of zones affected by plate edge	5 out of 69	5 out of 40
Number of wrong inhibition zones after adjustment	0 out of 69	2 out of 40
Number of wrong inhibition zones – Repeatability (25 measurements on same plate)	0 out of 125	Only done on a MH plate

5.1 Barcode/Label Scanning

All Barcodes were scanned and entered correctly in the system.

Acceptance Criteria of > 99% met.

5.2 Disk Recognition Accuracy

On the plates between 3 and 5 antibiotic disks were placed.

Total disks evaluated on 25 plates: 109

Correct automatic identification: 109

Accuracy: **100%**

Acceptance Criteria of > 99% met.

5.3 Zone Measurement Agreement

5.3.1 Study Design – 25 different agar plates

Automated Reading Only:

This is the values for the zones suggested by the PD-100 **without** adjustment:

- Overall agreement with manual reading within ±2 mm: **72%**
- By difficulty:
  - **MH plates:** 65 zones out of 69 zones were correct: **94%** (GREEN)
  - **MHF plates:** 13 zones out of 40 zones were correct: **33%** (GREEN)
- 3 of the 31 non-correct measurements were within ±3 mm (YELLOW)
- With **±3 mm** (see Ref. [3] and Ref. [5]) the overall agreement with manual reading is **74%**

Automated Reading After Operator Review:

This is the values for the zones after the operator has **reviewed and adjusted** in the PD-100:

- Overall agreement with manual reading within ±2 mm: **98%**
- By difficulty:
  - **MH plates:** 69 zones out of 69 zones were correct: **100%** (GREEN)
  - **MHF plates:** 38 zones out of 40 zones were correct: **95%** (GREEN)
- 1 of the 2 non-correct measurements were within ±3 mm (YELLOW), the last one was 4 mm apart, but still with the correct EUCAST interpretation
- With **±3 mm** (see Ref. [3] and Ref. [5]) the overall agreement with manual reading is **99%**

Acceptance Criteria of ≥ 90% within ±2 mm met for MH plates both before and after adjustment and for MHF plates after adjustment. All references found state that "*zones were adjusted if necessary*", see Ref. [3] – Ref. [6].

Bacteria Species:

The 25 plates represented 10 different bacteria species; the results give an indication of which bacteria are easy to measure and which are difficult to measure.

			Before adjustment		After adjustment	
Bacteria	**Plate type**	**Total Zones**	**Amount Zones correct**	**% Zones correct**	**Amount Zones correct**	**% Zones correct**
E. coli	MH	18	17	94%	18	100%
Klebsiella aerogenes	MH	9	9	100%	9	100%
Klebsiella oxytoca	MH	7	6	86%	7	100%
Proteus vulgaris	MH	7	6	86%	7	100%
S. aureus	MH	23	22	96%	23	100%
S. epidermidis[3]	MH	5	5	100%	5	100%
Haemophilus influenzae	MHF	25	3	12%	25	100%
Streptococcus dysgalactiae	MHF	8	6	75%	8	100%
Arcanobacterium haemolyticum[3]	MHF	3	2	67%	3	100%
Streptococcus pyogenes[3]	MHF	4	2	50%	2	50%
Total		**109**	**78**	**72%**	**107**	**98%**

For some of the bacteria, only one or two plates were available, which is insufficient to perform any meaningful statistical analysis. However, there is a clear tendency indicating that the MHF plates are the most challenging. Furthermore, *H. influenzae* often exhibits diffuse inhibition zones, making them difficult to measure both manually and automatically, as they require additional time and adjustment.

Typically, studies of automatic reading systems, see Ref. [3] – Ref. [6], only report performance after adjustment. All references found state that "*zones were adjusted if necessary*". It has been chosen to report all data in this study to provide the full transparency.

[3] Only 1 plate

5.3.2 Study Design – 25 measurements of the same agar plate

This is the values for the zones suggested by the PD-100 without adjustment:

- Overall agreement with manual reading within ±1 mm: **100%**

No adjustment needed by the Operator.

Acceptance Criteria of ≥ 95% within ±1 mm met.

5.4 Results Summary

Metric	Acceptance Criteria	Results	Fulfilled
Barcode/Label Scanning	> 99%	100%	YES
Disk Recognition Accuracy	> 99%	100%	YES
Categorical Agreement (CA)	≥ 90–95%	100%	YES
Zone Measurement Agreement **before** adjustment[4]	≥ 80%[4] within ±2 mm	MH plates: 94% MHF plates: 33% All plates: 72%	YES NO NO
Zone Measurement Agreement **after** adjustment	≥ 90% within ±2 mm	MH plates: 100% MHF plates: 95% All plates: 98%	YES YES YES
Zone Measurement Agreement for same plate – **Repeatability**	≥ 95% within ±1 mm	MH plate: 100%	YES
Very Major Error (VME)	≤ 1,5%	0	YES
Major Error (ME)	≤ 3%	0	YES

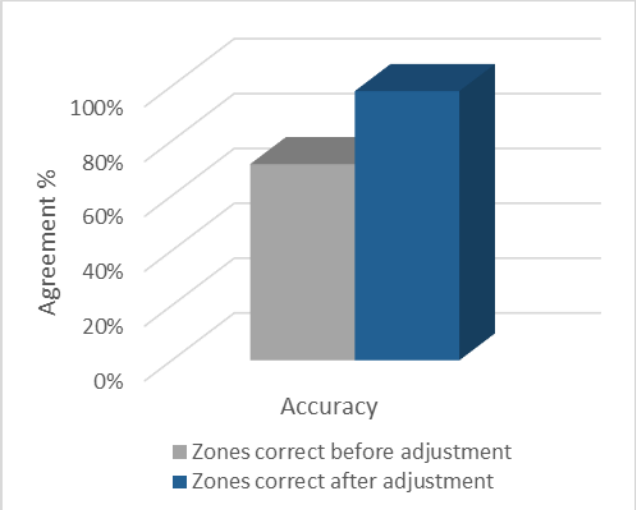

Figure 3 – Overall Agreement vs Manual Reference (%)

[4] No values found in the literature, all studies found only refers to acceptance **after** adjustment of zones

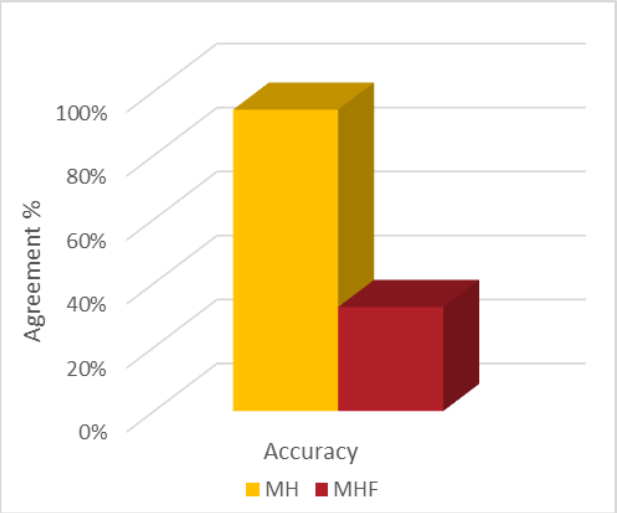

Figure 5 – Agreement by Plate before Adjustment (%)

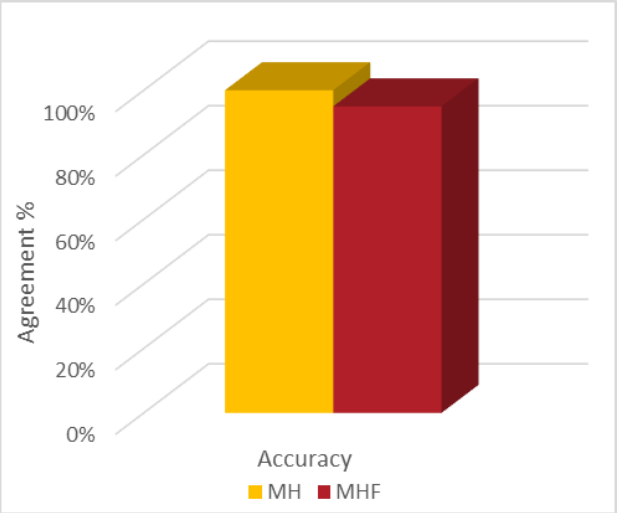

Figure 5 – Agreement by Plate After Operator Adjustment (%)

The plates selected for measurement were randomly chosen, with a particular focus on MHF plates, since these are the most challenging to interpret for the PD-100. The distribution was as follows:

- MH plates: 16 plates 64%
- MHF plates: 9 plates 36%

Typically, in the Microbiology Laboratory at Halland Sjukhus, MH plates account for 82% and MHF plates for 18%. Applying this standard distribution to the observed agreements yields these results:

- Observed overall agreement between PD-100 **before** adjustment and manual reference reading will be **83%** instead of 72%
- Observed overall agreement between PD-100 **after** operator verification and adjustment and manual reference reading will be **99%** instead of 98%.

6 Statistical Analysis

6.1 Improvement Analysis

Overall improvement after operator verification:

- Absolute improvement: 27%
- Relative improvement: 37%
- By difficulty:
  - **MH plates:**
    - Absolute improvement: 6%
    - Relative improvement: 6%
  - **MHF plates:**
    - Absolute improvement: 63%
    - Relative improvement: 192%
- Largest improvement observed in difficult plates (MHF plates)

6.2 Agreement Interpretation

Observed overall agreement between PD-100 before adjustment and manual reference reading was 72% and between PD-100 after operator verification and adjustment and manual reference reading was 98%.

Observed agreement indicates:

- High reproducibility vs manual reading
- No systematic bias toward over- or under-measurement
- Acceptable performance for routine diagnostic workflow

No evidence of systematic measurement drift was observed.

6.3 Bias Assessment

No directional bias was observed:

- No systematic overestimation of zone diameter
- No systematic underestimation of zone diameter
- Errors were randomly distributed across difficulty categories

This indicates algorithm neutrality relative to manual reading.

6.4 Reproducibility Assessment

Observed characteristics suggest high reproducibility due to:

- Standardized image-based measurement
- Automated edge detection algorithms
- Removal of inter-operator measurement variability
- Consistent illumination and measurement geometry
- Automated data transfer (no transcription errors)

Observed repeatability testing demonstrated 100% agreement within ±1 mm.

6.5 Clinical Risk Perspective

Risk of clinically relevant classification error expected to be low due to:

- High agreement with manual reference method
- Mandatory operator verification of zones
- Compliance with EUCAST interpretation workflow

7 Quality Assessment

7.1 EUCAST Compliance

- All inhibition zones measured
- Measurement resolution to nearest mm
- Calibration against manual reading
- Operator review possible for zones

7.2 Traceability & Documentation

- Automatic result transfer to LIMS
- Image storage facilitated traceability and allowed for retrospective analysis
- Reduced transcription error risk
- Improved compliance is a benefit for audits

8 Time Study and Resource Efficiency Analysis

A time study was performed to compare the operational efficiency of manual inhibition zone reading versus automated reading using the PD-100 system. The study evaluated total processing time per plate and associated staffing requirements.

Workflow Step	Manual Method	PD-100 Method
Zone Measurement	Manual measurement using calliper	Automatic image-based measurement
Data Entry	Manual entry into LIMS	Automatic data transfer to LIMS
Time noted from the plate was taken	..until the measurements were done and data typed	..and placed in the PD-100 and until it was taken out again, if adjustment was needed the time for this was noted separately

The time study evaluation is based on median processing times, which provide a robust and statistically appropriate central tendency measure for validation studies. Median values reduce the influence of extreme outliers and better represent typical routine laboratory workflow performance.

- Operational Efficiency Observations:
  - Manual workflow requires two trained laboratory staff (one for measurement and one for data entry) or longer time for one laboratory staff to make the data entry. Estimated time per plate for one operator including data entry is 40 seconds per plate[5].
  - The estimated time for one operator is used in the following comparison.
  - PD-100 workflow requires one trained operator with automated measurement and data transfer.
  - Operator intervention is only required for complex inhibition zones.
  - The comparison in time is only valid if the EUCAST guidelines are followed and all zones are measured.

Median Performance by Plate Type and overall – time measured in seconds:

Plate Type	Manual – Operators		PD-100			Difference to Manual 1 operator	Relative improvement
	2	1[5]	Automatic	Adjustment	Total time		
MH	30	40	15	2	17	23	57%
MHF	31	40	16	12	29	11	28%
All	31	40	15	6	21	19	47%

Applying the standard distribution between MH and MHF plates in the laboratory the overall relative time improvement will increase from 47% to 52%.

- Estimated Time Efficiency Impact:
  - Significant reduction in total processing time per plate using PD-100 compared to manual reading.
  - Reduction in manual transcription time and associated error risk.
  - Improved workflow consistency and reduced operator fatigue.
  - Improved laboratory throughput capacity.
  - Enables reallocation of laboratory personnel to higher value analytical tasks.

[5] Time for 1 operator for manual workflow is estimated by the Microbiology Laboratory in Halland Sjukhus

Operational Impact (100 plates/day):

- Time saved per day: appr. 31 minutes

The results demonstrate that PD-100 provides a consistent and measurable workflow efficiency improvement, particularly for moderate-difficulty (MH) plates and supports optimized laboratory resource utilization while maintaining EUCAST-compliant analytical performance. While the absolute time saving per plate is measured in seconds, the cumulative effect supports improved daily throughput, reduced manual handling, and enhanced operational consistency.

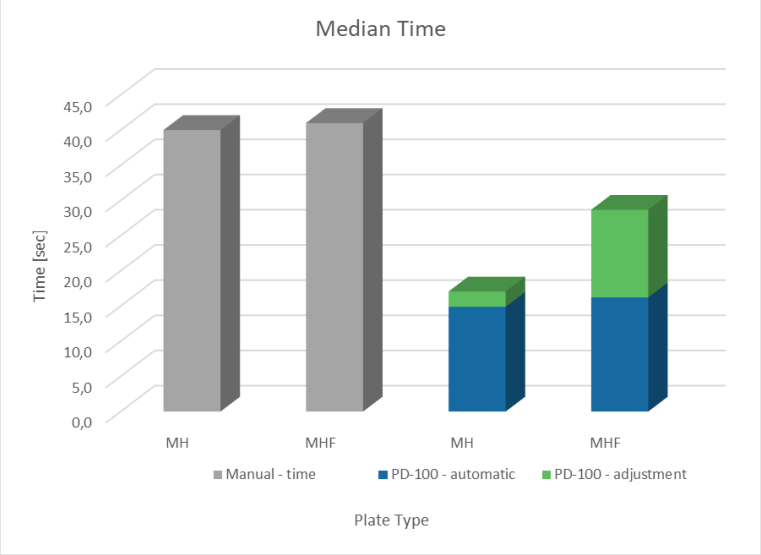

Figure 6 – Plate-Type Specific Time Comparison (Median Seconds)

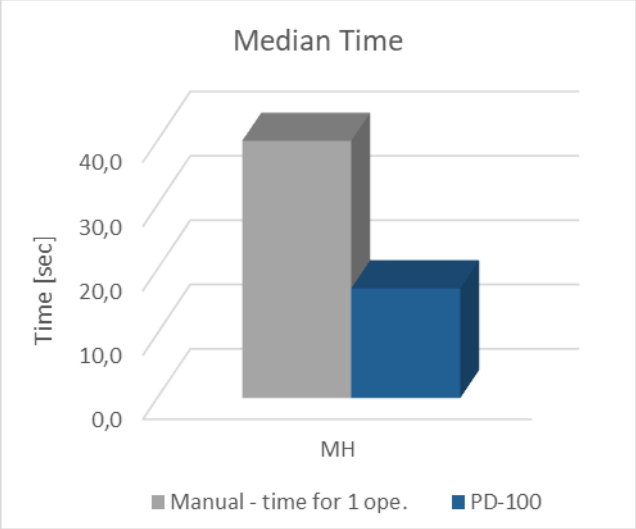

Figure 7 – MH Plates – Median Time in Seconds

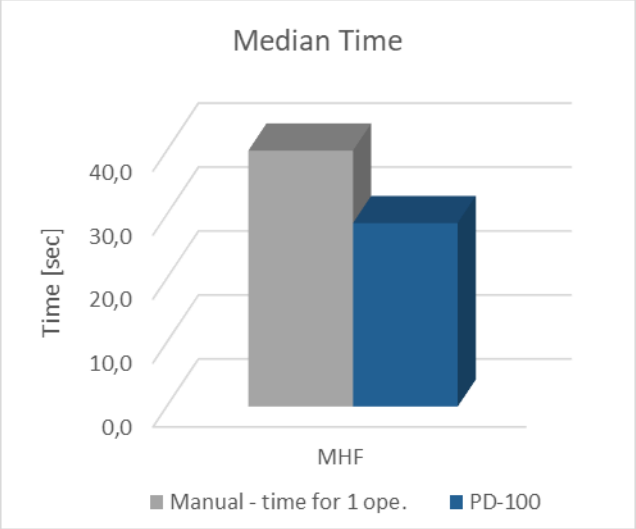

Figure 8 – MHF Plates – Median Time in Seconds

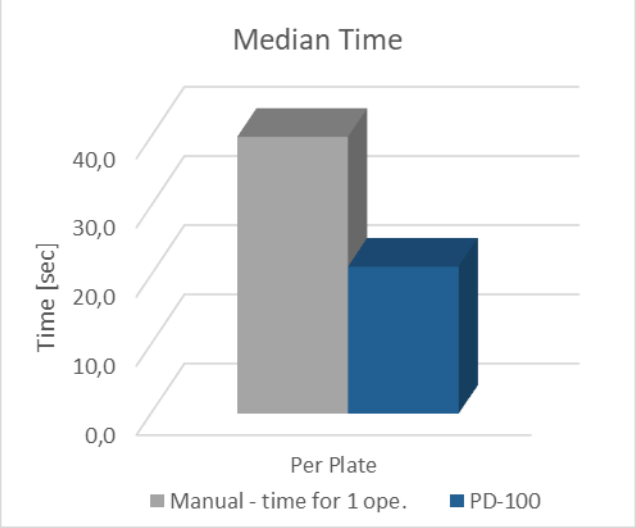

Figure 9 – All Plates – Median Time in Seconds

9 Conclusion

The validation study conducted at Hallands Sjukhus demonstrates that the PD-100 automated inhibition zone reader performs in analytical equivalence to manual EUCAST-compliant disk diffusion reading when used with standard operator verification.

The system achieved:

- **100%** disk recognition accuracy
- **98%** overall agreement with manual reference reading after operator verification
- **100%** categorical agreement (S/I/R interpretation)
- **100%** repeatability within ±1 mm in repeated measurements
- No very major errors (VME) or major errors (ME) observed

Performance on routine MH plates was consistently high, and while automated-only readings showed lower agreement for more complex MHF plates, operator verification effectively resolved discrepancies and ensured clinically reliable results.

No systematic measurement bias was observed, and no clinically significant deviations were identified during validation. The statistical evaluation supports that PD-100 provides robust, reproducible measurements suitable for routine diagnostic workflow under EUCAST methodology.

In addition to analytical performance, the time study demonstrated a significant and measurable workflow improvement. Using a realistic single-operator manual comparison, PD-100 reduced median processing time by up to 52% when adjusted to the laboratory's normal plate distribution, corresponding to approximately 31 minutes saved per 100 plates per day. The efficiency gain was most pronounced for routine MH plates.

Beyond time savings, PD-100 strengthens laboratory operations by:

- Enabling single-operator workflow
- Eliminating manual data transcription
- Standardizing zone measurement across operators
- Providing full image-based traceability
- Supporting audit readiness and documentation quality

Overall, the validation confirms that PD-100 delivers analytically reliable, EUCAST-compliant inhibition zone measurement while improving workflow efficiency, consistency, and traceability under routine laboratory conditions.

10 References

Document	
Ref. [1]	Antimicrobial Susceptibility Testing – EUCAST Disk Diffusion Method, Version 13.0, January 2025. Available at: https://www.eucast.org
Ref. [2]	EUCAST Breakpoint tables for interpretation of MICs and zone diameters, Version 16.0, valid from 2026-01-01
Ref. [3]	Evaluation of the Oxoid Aura image system for measuring zones of inhibition with the disc diffusion technique – J. M. Andrews, F. J. Boswell and R. Wise – Department of Microbiology, City Hospital NHS Trust, Birmingham B18 7QH, UK – Journal of Antimicrobial Chemotherapy (2000) 46, p535–540
Ref. [4]	Evaluation of an Automated System for Reading and Interpreting Disk Diffusion Antimicrobial Susceptibility Testing of Fastidious Bacteria, Evgeny A. Idelevich, Karsten Becker, Janne Schmitz, Dennis Knaack, Georg Peters, Robin Köck – University Hospital Münster, Münster, Germany – PLOS One, July 2016, Vol. 7
Ref. [5]	Comparison and Evaluation of Osiris and Sirscan 2000 Antimicrobial Susceptibility Systems in the Clinical Microbiology Laboratory – A. Nijs, R. Cartuyvels, A. Mewis, V. Peeters, J. L. Rummens, and K. Magerman – Department of Clinical Laboratory, Virga Jesseziekenhuis, Belgium – JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2003, p. 3627–3630 Vol. 41, No. 8
Ref. [6]	Comparison of the Copan WASPLab incorporating the BioRad expert system against the SIRscan 2000 automatic for routine antimicrobial disc diffusion susceptibility testing – A. Cherkaoui, G. Renzi, A. Fischer, N. Azam, D. Schorderet, N. Vuilleumier, J. Schrenzel – Clinical Microbiology and Infection 26 (2020), p619-625
Ref. [7]	ISO 20776-2:2021 – Clinical laboratory testing and in vitro diagnostic test systems – Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices