PhenoMATRIX®: can Artificial do better than Human Intelligence?

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INTRODUCTION

Traditional culture techniques and the adoption of agar plates are still widely used within the field of diagnostic microbiology. The assessment of the live microbial cell content by visual examination of their growth remains an essential tool in diagnosing bacterial infections from various specimens.

All the methods based on visual examinations are inevitably subjective, and, in most cases, they are qualitative because quantitative approaches are time and labor-consuming. Hence, image analysis has become fundamental to streamlining the workflow and increasing laboratory efficiency since its adoption.

PhenoMATRIX® is one of the multiple Copan solutions to speed up the time-to-result. Using specific AI-based algorithms to interpret microbial growth, the software classifies and sorts the images of the culture plates incubated in WASPLab, allowing the user to quickly report the homogenous results created by the system.

The assessment of PhenoMATRIX® performance is one of the problems that come to Copan every day before the release of each meticulously developed software customization. The definition of its analytical and clinical performance is complicated by the lack of a gold standard for the interpretation of microbial growth on culture plates, which is based on visual examination and sometimes fails when it comes to reproducibility. In this context, **the usual performance indicators have changed their definition**:

- accuracy of PhenoMATRIX® analysis becomes the agreement between the **software classification** and the **lab technologist reading**,
- sensitivity and specificity are calculated respectively as positive and negative percent agreement with the technologist's interpretation.

However, the methods that Copan established for software development and performance evaluation recently obtained one important acknowledgment: PhenoMATRIX® has been certified as Class C in-vitro diagnostic medical device in accordance with the IVDR 2017/746.

HOW DOES IT WORK?

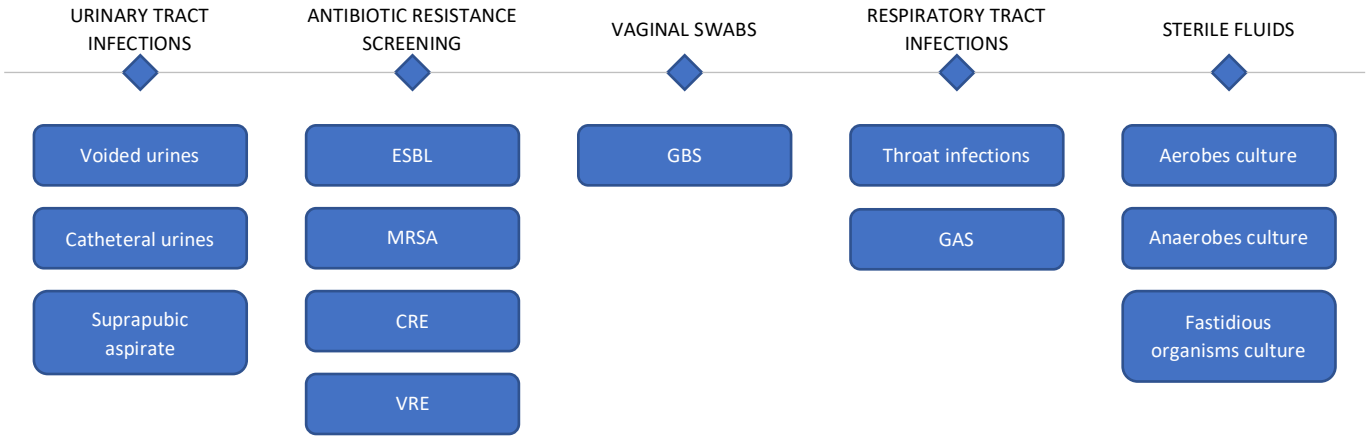
The external appearance of the colonies grown on agar substrate, including color, texture, shape, and surface aspect – as well as their reaction with the culture medium – are among the essential phenotypic characteristics in the microbial "presumptive" identification performed by the microbiology experts during routinary analysis for differentiating microorganisms at the level of various taxonomic groups.

PhenoMATRIX® does the same. The software scans the culture plate image looking for microbial colonies and, whenever found, guesses which the responsible organism is based on color, morphology, β-hemolysis, or a combination of the three. The ability to detect specific features of microbial growth depends on the knowledge gained during its training, which is performed by showing to the image analysis algorithms several images of various colonies grown on different substrates. PhenoMATRIX® *Artificial Intelligence* (AI) capabilities also include the semiquantitative assessment of the microbial load and the determination of the culture purity.

Table 1. Technical characteristics of the WASPLab imaging system

Imaging System Technology	
Camera	**48MPX** (16*3) - Camera RGB (Red/ Green/Blue)
Depth of field	**9mm**
Resolution	0,026mm/RGB pixel - 26µm/RGB pixel - **4800 pixel (1600 RGB pixel)/ 1mm²**
Minimum colony size	**<0,1mm**
Color depth	**24 BIT**

Figure 1. List of PhenoMATRIX current applications



In combination with the Image Analysis algorithms, PhenoMATRIX® takes advantage of the patient clinical and demographic data to direct the culture plates to the appropriate grouping.

The software interface is built to show all culture analysis protocols on one page and divided into subfolders, where plate images are sorted by PhenoMATRIX® rules to send all results of the folder in batch with one click.

TAILORED

PhenoMATRIX® is always designed and configured for the needs of each specific customer. Its Artificial Intelligence based on *Neural Networks* is trained by the Copan WASP Computer Vision team, composed of 15 highly specialized professionals dedicated full-time to digital imaging and analysis. The team takes care of each product – software and algorithms - from R&D to the perpetual development and improvement through the product's entire lifecycle.

The fully customizable expert rules implemented in PhenoMATRIX® can use a large variety of data, including:

- Demographic data (e.g., gender, age of the patient).
- Clinical and analytical data (e.g., results of collateral clinical analyses or presence of pathologies);
- Incubation information (final vs. intermediate time).
- Semiquantitative assessment of the microbial growth present on the culture plate.
- Presumptive identification of pathogens or classes of pathogens by color and/or morphology on the culture plate.
- Predominance of target pathogens over other microorganisms.
- Detection of β-hemolysis.

Furthermore, PhenoMATRIX® can apply the result on every single plate or review all the plates belonging to the same sample, combining their interpretation to provide a final result for the analysis ("multiplate" mode).

Since mid-2018, PhenoMATRIX® has been successfully implemented with more than **75** different protocol configurations belonging to the most common applications (Figure 1).

Figure 2. Example of PhenoMATRIX® interface, with the images of urine cultures with a suspected presence of gram-negative microorganisms, sorted in one single folder

PERFORMANCE EVALUATION – TWO CASE STUDIES

Urinary Tract Infections (UTI) and early reading of culture plates

The most common application of PhenoMATRIX® is the analysis of urine cultures after 16-24 hours of incubation. A multitude of laboratories daily uses PhenoMATRIX® to interpret urine culture plates and validation studies aimed to compare the classification attributed by PhenoMATRIX® to the interpretation of the lab technologist. Table 2 summarizes raw data collected during performance studies on **33.104** urine samples from **16 laboratories**. Since each laboratory has customized expert rules to sort plates images in personalized folders with tailored results, a considerable effort was made for the aggregation of the heterogeneous results into four major categories:

- Negatives/Non-significant (**N**, <10^3 CFU/mL in urine)
- Positives with *Escherichia coli* (**P/EC**, ≥10^3 CFU/mL with *E. coli* predominance)
- Positives with other organisms (**P/OO**, ≥10^3 CFU/mL with other pathogens predominance)
- Contamination/Normal Flora (**C**, ≥10^3 CFU/mL with three or more organisms)

Table 2. Raw contingency table summarizing cultures interpretation results

			Visual interpretation				
			N	P		C	∑
				EC	OO		
PhenoMATRIX®		N	17614	0	5	88	17707
	P	EC	24	5133	7	100	5264
		OO	554	44	2775	1150	4523
		C	359	23	83	5145	5610
		∑	18551	5200	2870	6483	33104

These studies demonstrated a very high agreement between PhenoMATRIX® and laboratory interpretation of urine cultures, specifically regarding positive and negative plates which account for up to 80-90% of the plates volume (Table 3), with the accuracy calculated as the percentage of plates of the clinical category which were interpreted by PhenoMATRIX® in agreement with the lab technologist.

Table 3. PhenoMATRIX® performance on urine cultures incubated for 16-24 hours.

Clinical category	Estimated occurrence	% accuracy[1]
Negatives/Non-significant (N)	40-50%	94,9% (specificity)
All positives (P)	30-40%	98,9% (sensitivity)
Positives with *E. coli* (EC)	10-20%	98,7%
Positives with other organisms (OO)	10-20%	96,7%
Contamination/Normal Flora (C)	10-20%	79,4%

The analysis of discrepant results that were interpreted as negative/non-significant by PhenoMATRIX® showed that the software output is more rigorous than the visual interpretation of the lab technologist, especially on those plates with uncertain results, e.g., with the number of colonies near the "non-significant-growth threshold" or with the presence of microcolonies in small quantity. PhenoMATRIX® indeed strictly applies the interpretation rules that were agreed upon with the laboratory and is never affected by subjectivity. The software output is more reproducible than the visual interpretation.

The high sensitivity of PhenoMATRIX® and the possibility to take pictures of culture plates at multiple incubation times become the preconditions for the early reading of culture plates (≤12 hours of incubation), specifically for the detection of *E. coli* and other uropathogens on chromogenic plates. PhenoMATRIX® algorithms have been fine-tuned using images of colonies at 8 to 12 hours incubation and tested compared to the visual reading of the culture plate images. The software sensitivity and specificity regarding the presence of the target organisms at 8 hours of incubation are reported in Table 4. Performance increases at 12 hours of incubation due to the more pronounced reaction of the chromogenic substrate.

Table 4. PhenoMATRIX® performance on early detection (8 hours) of uropathogenic organisms

Organisms	Sensitivity	Specificity
E. coli	96,2%	93,7%
KESC	95,8%	75,1%
PMP	100%	89,3%

[1] $\%\ A = \frac{no.\ plates\ confirmed\ by\ lab\ tech\ (software\ agreement)}{total\ no.\ of\ plates\ classified\ as\ N/EC/OO/C\ by\ the\ lab\ tech}$

Sterile body fluids and the management of multiple analyses

The analysis of wound swabs, blood, and body cavity fluids usually requires the review of multiple culture plates inoculated from the same specimen, making the reporting excessively burdensome, in consideration of the expectation that the majority of specimens are sterile.

PhenoMATRIX® "multiplate" feature permits the simultaneous evaluation of all the plates relevant to the one sample, overcoming the issue by also facilitating the review for the operator.

Currently, PhenoMATRIX® "multiplate" protocols are applied to urine culture and sterile body fluids over different analyses, for the recovery of gram-positive and gram-negative aerobes, fastidious organisms, and even anaerobes.

PhenoMATRIX® displays in the same interface the culture plates inoculated from the same samples (for example, *Columbia CNA agar + 5% sheep blood/Chocolate agar/MacConkey* or *Columbia agar + 5% sheep blood/Chocolate agar/Schaedler agar+ 5% sheep blood*). When all the plates do not show any microbial growth, PhenoMATRIX® reports the entire sample as negative at the end of incubation; otherwise, it can suggest a longer incubation or let the operator review the microbial growth even at a shorter incubation time. With 100% sensitivity and 99,3% specificity, determined on 2.462 samples, the "multiplate" image analysis permits a quick reporting of the negative samples, accounting for approximately 67% of body fluids specimens fully managed through PhenoMATRIX®.

Improved TAT and cost reduction

Several articles have been published on the benefits of Copan WASP AI-integrated automation on time to results and labor savings.

Faron et al. [1] evaluated the improvement of TAT for urine samples before and after the use of the WASPLab coupled with Copan WASP image analysis software, reporting a median reduction of 3 h 28 min for positive specimens and 4 h 42 min for negative specimens. The automatic analysis performed by software indeed anticipates the screening of all the negative and positive plates that require no additional workup for reporting, which can be released without manual processing.

In another study, [2] the researchers estimated the potential savings in terms of labor cost per specimen in a VRE screening protocol that could achieve a 79% reduction for the negative samples. The cost reduction was estimated by considering that it took 9,6 min of a technologist's time to manually process, read, and report each negative VRE specimen, while fully automated processing with the use of image analysis would require only 2 min of hands-on time.

Culbreath [3] investigated both the cost reduction and productivity improvement in four laboratories that adopted Copan WASP full laboratory automation, three of which also purchased the image analysis software modules. The major benefits were for the laboratory that implemented the image analysis on multiple protocols that included 80% of their cultures, with up to 13,6 full-time equivalent (FTE) cost avoidance compared to the manual workflow, thanks to the reduction in time to screen their large volume of negative cultures. The average cost reduction per specimen varies from 15% to 38%, also based on the labor cost of each laboratory. The study also reported an improvement in TAT, with a median reduction of 14 hours from the manual to the fully automated workflow, 8 of which are attributed to the implementation of image analysis algorithms.

Conclusions

Copan's Workflow-Integrated System Environment (WISE) provides solutions from sample collection, to transport, processing, and interpretation. PhenoMATRIX® finds its place in the last step of this workflow: its advanced software, AI systems, and interpretation algorithms have been developed to maximize lab efficiency and support diagnostic decisions, improving treatment indication and patient outcomes.

Thanks to the accurate interpretation and tailored configuration, PhenoMATRIX® is the tool to report all the results quickly. The possibility to review all plates in batches, automatically reporting to the LIS the final or intermediate results, allows for the total automatization of those plates that do not require further workups before reporting.

For urine samples, the volume of negatives accounts for approximately 50% of plates, for which hands-on time becomes null with PhenoMATRIX®, which accurately classifies 94,9% of plates. Specifically, 99,5% (17614/17707) of plates images in the PhenoMATRIX® folder for negative plates are confirmed by the lab technologist and do not need any action during the

review. The reproducibility of PhenoMATRIX® interpretation is superior to the human reading due to the rigorous application of thresholds and expert rules. Among the positives, chromogenic plates with E. coli (about 15%) can be directly reported and, with the optional feature of colony tagging of PhenoMATRIX® TAG coupled with Colibrí™, also these plates can be fully automatically processed, removing around 65% of urine cultures from the laboratory manual workflow.

A further advantage of using PhenoMATRIX® is the possibility to schedule the analysis of the microbial growth at early incubation. Thanks to the high sensitivity of image analysis to the color development in chromogenic plates, the presence of significant microorganisms can be reported early to the microbiologist and analyzed in advance, improving turnaround time (TAT). Even though scientific evidence shall be provided on the possibility of reporting a urine culture as early as after 8 hours of incubation, PhenoMATRIX® demonstrated high sensitivity and good specificity in detecting common uropathogens on chromogenic plates that can be used for a faster Time to Result.

The “multiplate” mode is another essential feature for reducing the burden of reporting a sample with multiple analyses. By sorting the plate images belonging to the same sample to be displayed in groups, PhenoMATRIX® provides a clear visualization that facilitates and accelerates the user review. Also, the simultaneous plates assessment with the generation of a common result, thanks to the high accuracy of image analysis, allows for the creation of batches of negative samples that can be quickly reported saving the microbiologist’s expertise for more critical samples.

The benefits of PhenoMATRIX® include the cost saving of plate reporting [2] as well as the TAT reduction, as the accurate plate batching allows for the efficient release of no growth and insignificant cultures, permitting the work on more significant cultures to begin sooner. This can significantly reduce the workload in the laboratory and allow physicians to make informed treatment decisions earlier [1].

Although the interpretation of some samples still does not have strict and precise quantitative criteria that can be fixed in expert rules, PhenoMATRIX® can offer the advantage of reproducibility and the possibility to be fine-tuned, overcoming the subjectivity of the human being in order to more accurately investigate the microbial content of samples.

References

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