specific technologies

Addendum to the publication

Colorimetric Sensor Array Allows Fast Detection and Simultaneous Identification of Sepsis-Causing Bacteria in Spiked Blood Culture

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1 Executive Summary

This addendum describes the application of a more refined classification strategy developed for the utilization of the Colorimetric Sensor Array (CSA) data described in above-mentioned publication. The underlying data, all compared to BacT/ALERT™, is unchanged, but here instead of using a flat classifier separating all bacterial species at once a hierarchical system was developed, first distinguishing between Gram-negative and Gram-positive microorganisms, then separately classifying species of both Gram states in a second level classifier.

We here report, for the first time, 99.4% accurate classification of Gram status immediately at the time point of detection. Further, the results reveal improved classification performance for a panel of 18 bacterial species commonly present in clinical environments. In summary, overall classification accuracy (calculated using micro-averaging for the cascading classifier) improved from 83.7% to now 86.2% at time of detection, and from 91.9% to now 94.0% at 2 hours after detection.

Finally, while detection times here averaged 3.0 hours faster than those obtained using the BacT/ALERT™ CO_2 indicator, we found that the detection algorithm utilized in the paper was very conservative. Figure 1 illustrates, that the sensor responses indicating positive sample emerged several hours earlier than the detection algorithm used in the publication indicates. A second revision of this work using a newly developed positive detection algorithm will be distributed subsequently.

The body of the report below present confusion matrices and performance for classifiers on both levels of the hierarchical system individually for all 18 species tested.

2 Top Level Classification (Gram Status)

2.1 At time of detection

Using repeated (10x), stratified cross-validation, a classifier achieved an overall accuracy of **99.42%** with a 95% confidence interval ranging from **99.27%** to **99.55%** in the discrimination of gram-positive and gram-negative bacteria as well as non-inoculated controls.

	Control	gram-	gram+
Control	1030	0	0
gram-	0	5061	39
gram+	10	20	5760

Table 1: Confusion Matrix, at time of detection

	Bal.	Sensitivity	Specificity	PPV	NPV
Control	99.52	99.04	100.00	100.00	99.91
gram-	99.52	99.61	99.43	99.24	99.71
gram+	99.42	99.33	99.51	99.48	99.36

Table 2: Classification metrics [%] at time of detection

2.2 At time of detection +2h

Using repeated (10x), stratified cross-validation, a classifier achieved an overall accuracy of **99.44%** with a **95%** confidence interval ranging from **99.29%** to **99.56%** in the discrimination of gram-positive and gram-negative bacteria as well as non-inoculated controls.

	Control	gram-	gram+
Control	1030	0	0
gram-	0	5055	45
gram+	0	22	5768

Table 3: Confusion Matrix, at time of detection +2h

	Bal.	Sensitivity	Specificity	PPV	NPV
Control	100.00	100.00	100.00	100.00	100.00
gram-	99.45	99.57	99.34	99.12	99.68
gram+	99.43	99.23	99.64	99.62	99.27

Table 4: Classification metrics [%] at t=2h

3 Sub-level Classification (Gram Positive Species ID)

3.1 At time of detection

Using repeated (5x), stratified cross-validation, a classifier achieved an overall accuracy of **86.36%** with a 95% confidence interval ranging from **85.05%** to **87.59%** in the discrimination of gram+ species.

	Enterococcus faecalis	*Enterococcus faecium*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Staphylococcus lugdunensis*	*Streptococcus agalactiae*	*Streptococcus pneumoniae*	*Streptococcus pyogenes*
Enterococcus faecalis	313	25	4	4	1	18	0	15
Enterococcus faecium	24	312	1	2	0	6	5	0
Staphylococcus aureus	9	4	564	15	6	12	6	14
Staphylococcus epidermidis	3	2	30	218	2	0	0	20
Staphylococcus lugdunensis	0	0	11	1	230	0	2	1
Streptococcus agalactiae	13	0	24	0	0	233	0	10
Streptococcus pneumoniae	0	1	20	12	0	5	355	2
Streptococcus pyogenes	14	4	25	19	0	3	0	275

Table 5: Confusion Matrix for gram+ species at time of detection

	Bal.	Sensitivity	Specificity	PPV	NPV
Enterococcus faecalis	90.29	83.24	97.34	82.37	97.50
Enterococcus faecium	94.08	89.66	98.51	89.14	98.59
Staphylococcus aureus	90.04	83.06	97.02	89.52	94.92
Staphylococcus epidermidis	89.14	80.44	97.83	79.27	97.98
Staphylococcus lugdunensis	97.83	96.23	99.44	93.88	99.66
Streptococcus agalactiae	91.16	84.12	98.20	83.21	98.32
Streptococcus pneumoniae	97.44	96.47	98.42	89.87	99.48
Streptococcus pyogenes	89.53	81.60	97.46	80.88	97.57

Table 6: Classification metrics [%] for gram+ species at time of detection

3.2 At time of detection +2 hours

Using repeated (5x), stratified cross-validation, a classifier achieved an overall accuracy of **93.89%** with a 95% confidence interval ranging from **92.95%** to **94.73%** in the discrimination of gram+ species.

	Enterococcus faecalis	*Enterococcus faecium*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Staphylococcus lugdunensis*	*Streptococcus agalactiae*	*Streptococcus pneumoniae*	*Streptococcus pyogenes*
Enterococcus faecalis	347	12	1	0	0	8	0	12
Enterococcus faecium	18	317	0	0	0	5	10	0
Staphylococcus aureus	3	2	607	3	0	4	6	5
Staphylococcus epidermidis	3	0	8	260	1	0	0	3
Staphylococcus lugdunensis	0	0	0	6	234	5	0	0
Streptococcus agalactiae	3	0	18	0	0	249	0	10
Streptococcus pneumoniae	0	0	4	0	0	4	383	4
Streptococcus pyogenes	4	3	5	0	0	5	2	321

Table 7: Confusion Matrix for gram+ species at time of detection +2h

	Bal.	Sensitivity	Specificity	PPV	NPV
Enterococcus faecalis	95.24	91.80	98.69	91.32	98.77
Enterococcus faecium	96.81	94.91	98.71	90.57	99.33
Staphylococcus aureus	96.69	94.40	98.98	96.35	98.41
Staphylococcus epidermidis	98.04	96.65	99.43	94.55	99.66
Staphylococcus lugdunensis	99.58	99.57	99.59	95.51	99.96
Streptococcus agalactiae	93.87	88.93	98.81	88.93	98.81
Streptococcus pneumoniae	97.52	95.51	99.52	96.96	99.28
Streptococcus pyogenes	94.84	90.42	99.25	94.41	98.67

Table 8: Classification metrics [%] at t=2h

4. Sub-level Classification (Gram Negative Species ID)

4.1 At time of detection

Using repeated (5x), stratified cross-validation, a classifier achieved an overall accuracy of **83.29%** with a 95% confidence interval ranging from **81.79%** to **84.72%** in the discrimination of gram- species.

	Acinetobacter baumannii	*Enterobacter aerogenes*	*Enterobacter cloacae*	*Escherichia coli*	*Klebsiella oxytoca*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudmonas aeruginos*	*Salmonella enterica*	*Serratia marcescens*
Acinetobacter baumannii	235	0	0	1	0	5	0	5	4	0
Enterobacter aerogenes	0	156	4	7	44	14	0	0	0	0
Enterobacter cloacae	0	8	101	17	8	7	5	0	11	8
Escherichia coli	0	15	0	288	0	20	5	0	7	0
Klebsiella oxytoca	0	54	1	3	169	16	0	0	2	0
Klebsiella pneumoniae	0	16	4	20	14	284	0	0	2	0
Proteus mirabilis	5	0	0	0	0	0	243	7	0	5
Pseudomonas aeruginosa	0	0	0	0	0	0	0	235	0	0
Salmonella enterica	2	1	14	10	5	2	11	0	218	7
Serratia marcescens	0	0	18	7	0	0	0	0	5	195

Table 9: Confusion Matrix for gram- species at time of detection

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
Acinetobacter baumannii	98.23	97.11	99.35	94.00	99.70
Enterobacter aerogenes	79.70	62.40	97.00	69.33	95.96
Enterobacter cloacae	84.23	71.13	97.34	61.21	98.28
Escherichia coli	89.72	81.59	97.86	85.97	97.07
Klebsiella oxytoca	83.56	70.42	96.71	68.98	96.92
Klebsiella pneumoniae	89.53	81.61	97.46	83.53	97.10
Proteus mirabilis	95.65	92.05	99.26	93.46	99.08
Pseudomonas aeruginosa	97.57	95.14	100.00	100.00	99.48
Salmonella enterica	92.65	87.55	97.74	80.74	98.64
Serratia marcescens	94.71	90.70	98.72	86.67	99.14

Table 10: Classification metrics [%]at time of detection

4.2 At time of detection +2h

Using repeated (5x), stratified cross-validation, a classifier achieved an overall accuracy of **92.86%** with a 95% confidence interval ranging from **91.79%** to **93.83%** in the discrimination of gram- species.

	Acinetobacter baumannii	*Enterobacter aerogenes*	*Enterobacter cloacae*	*Escherichia coli*	*Klebsiella oxytoca*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Salmonella enterica*	*Serratia marcescens*
Acinetobacter	250	0	0	0	0	0	0	0	0	0
Enterobacter	0	196	0	0	26	3	0	0	0	0
Enterobacter cloacae	0	0	133	18	6	0	3	0	0	5
Escherichia coli	0	4	4	298	5	10	5	0	3	6
Klebsiella oxytoca	0	20	11	0	214	0	0	0	0	0
Klebsiella pneumoniae	0	14	0	12	7	307	0	0	0	0
Proteus mirabilis	0	0	0	0	0	0	260	0	0	0
Pseudomonas	0	0	0	0	0	0	0	235	0	0
Salmonella enterica	0	0	0	0	5	0	9	0	251	5
Serratia marcescens	0	0	1	0	0	0	0	0	0	224

Table 11: Confusion Matrix for gram- species at time of detection +2h

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
Acinetobacter baumannii	100.00	100.00	100.00	100.00	100.00
Enterobacter aerogenes	91.25	83.76	98.75	87.11	98.37
Enterobacter cloacae	93.96	89.26	98.67	80.61	99.33
Escherichia coli	94.59	90.85	98.33	88.96	98.65
Klebsiella oxytoca	90.01	81.37	98.64	87.35	97.87
Klebsiella pneumoniae	97.23	95.94	98.52	90.29	99.41
Proteus mirabilis	96.93	93.86	100.00	100.00	99.26
Pseudomonas aeruginosa	100.00	100.00	100.00	100.00	100.00
Salmonella enterica	99.00	98.82	99.17	92.96	99.87
Serratia marcescens	96.65	93.33	99.96	99.56	99.31

Table 12: Classification metrics [%] at t=2h

5 . Time to Detection vs. BacT/ALERT™

5.1 Gram+ species

5.1.1 *Enterococcus faecalis*

Using the CSA paradigm growth was detected after **10.55h** compared to **12.53h** using BacT/ALERT™, resulting in an average advantage of **1.98h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	90.29	83.24	97.34	82.37	97.50
time of detection +2h	95.24	91.80	98.69	91.32	98.77

Table 13: Time-resolved classification performance for *Enterococcus faecalis* [%]

5.1.2 *Enterococcus faecium*

Using the CSA paradigm growth was detected after **14.24h** compared to **18.46h** using BacT/ALERT™, resulting in an average advantage of **4.22h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	94.08	89.66	98.51	89.14	98.59
time of detection +2h	96.81	94.91	98.71	90.57	99.33

Table 14: Time-resolved classification performance for *Enterococcus faecium* [%]

5.1.3 *Staphylococcus aureus*

Using the CSA paradigm growth was detected after **12.43h** compared to **14.85h** using BacT/ALERT™, resulting in an average advantage of **2.42h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	90.04	83.06	97.02	89.52	94.92
time of detection +2h	96.69	94.40	98.98	96.35	98.41

Table 15: Time-resolved classification performance for *Staphylococcus aureus* [%]

5.1.4 *Staphylococcus epidermidis*

Using the CSA paradigm growth was detected after **15.44h** compared to **20.71h** using BacT/ALERT™, resulting in an average advantage of **5.27h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	89.14	80.44	97.83	79.27	97.98
time of detection +2h	98.04	96.65	99.43	94.55	99.66

Table 16: Time-resolved classification performance for *Staphylococcus epidermidis* [%]

5.1.5 *Staphylococcus lugdunensis*

Using the CSA paradigm growth was detected after **16.38h** compared to **22.50h** using BacT/ALERT™, resulting in an average advantage of **6.12h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	97.83	96.23	99.44	93.88	99.66
time of detection +2h	99.58	99.57	99.59	95.51	99.96

Table 17: Time-resolved classification performance for *Staphylococcus lugdunensis* [%]

5.1.6 *Streptococcus agalactiae*

Using the CSA paradigm growth was detected after **9.66h** compared to **11.84h** using BacT/ALERT™, resulting in an average advantage of **2.18h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	91.16	84.12	98.20	83.21	98.32
time of detection +2h	93.87	88.93	98.81	88.93	98.81

Table 18: Time-resolved classification performance for *Streptococcus agalactiae* [%]

5.1.7 *Streptococcus pneumoniae*

Using the CSA paradigm growth was detected after **13.18h** compared to **16.48h** using BacT/ALERT™, resulting in an average advantage of **3.30h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	97.44	96.47	98.42	89.87	99.48
time of detection +2h	97.52	95.51	99.52	96.96	99.28

Table 19: Time-resolved classification performance *for Streptococcus pneumoniae* [%]

5.1.8 *Streptococcus pyogenes*

Using the CSA paradigm growth was detected after **11.85h** compared to **16.00h** using BacT/ALERT™, resulting in an average advantage of **4.15h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	89.53	81.60	97.46	80.88	97.57
time of detection +2h	94.84	90.42	99.25	94.41	98.67

Table 20: Time-resolved classification performance for *Streptococcus pyogenes* [%]

5.2 Gram- organisms

5.2.1 *Acinetobacter baumannii*

Using the CSA paradigm growth was detected after **12.83h** compared to **12.13h** using BacT/ALERT™, resulting in an average disadvantage of **-0.70h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	98.23	97.11	99.35	94.00	99.70
time of detection +2h	100.00	100.00	100.00	100.00	100.00

Table 21: Time-resolved classification performance for *Acinetobacter baumannii* [%]

5.2.2 *Enterobacter aerogenes*

Using the CSA paradigm growth was detected after **11.60h** compared to **12.65h** using BacT/ALERT™, resulting in an average advantage of **1.05h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	79.70	62.40	97.00	69.33	95.96
time of detection +2h	91.25	83.76	98.75	87.11	98.37

Table 22: Time-resolved classification performance for *Enterobacter aerogenes* [%]

5.2.3 *Enterobacter cloacae*

Using the CSA paradigm growth was detected after **10.49h** compared to **13.32h** using BacT/ALERT™, resulting in an average advantage of **2.83h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	84.23	71.13	97.34	61.21	98.28
time of detection +2h	93.96	89.26	98.67	80.61	99.33

Table 23: Time-resolved classification performance for *Enterobacter cloacae* [%]

5.2.4 *Escherichia coli*

Using the CSA paradigm growth was detected after **10.12h** compared to **13.01h** using BacT/ALERT™, resulting in an average advantage of **2.89h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	89.72	81.59	97.86	85.97	97.07
time of detection +2h	94.59	90.85	98.33	88.96	98.65

Table 24: Time-resolved classification performance for *Escherichia coli* [%]

5.2.5 *Klebsiella oxytoca*

Using the CSA paradigm growth was detected after **10.92h** compared to **13.12h** using BacT/ALERT™, resulting in an average advantage of **2.20h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	83.56	70.42	96.71	68.98	96.92
time of detection +2h	90.01	81.37	98.64	87.35	97.87

Table 25: Time-resolved classification performance for *Klebsiella oxytoca* [%]

5.2.6 *Klebsiella pneumoniae*

Using the CSA paradigm growth was detected after **9.68h** compared to **12.00h** using BacT/ALERT™, resulting in an average advantage of **2.32h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
	89.53	81.61	97.46	83.53	97.10
time of detection +2h	97.23	95.94	98.52	90.29	99.41

Table 26: Time-resolved classification performance for *Klebsiella pneumoniae* [%]

5.2.7 *Proteus mirabilis*

Using the CSA paradigm growth was detected after **10.47h** compared to **12.78h** using BacT/ALERT™, resulting in an average advantage of **2.31h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	95.65	92.05	99.26	93.46	99.08
time of detection +2h	96.93	93.86	100.00	100.00	99.26

Table 27: Time-resolved classification performance for *Proteus mirabilis* [%]

5.2.8 *Pseudomonas aeruginosa*

Using the CSA paradigm growth was detected after **15.79h** compared to **17.05h** using BacT/ALERT™, resulting in an average advantage of **1.26h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	97.57	95.14	100.00	100.00	99.48
time of detection +2h	100.00	100.00	100.00	100.00	100.00

Table 28: Time-resolved classification performance for *Pseudomonas aeruginosa* [%]

5.2.9 *Salmonella enterica*

Using the CSA paradigm growth was detected after **10.66h** compared to **15.65h** using BacT/ALERT™, resulting in an average advantage of **4.99h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	92.65	87.55	97.74	80.74	98.64
time of detection +2h	99.00	98.82	99.17	92.96	99.87

Table 29: Time-resolved classification performance for *Salmonella enterica* [%]

5.2.10 *Serratia marcescens*

Using the CSA paradigm growth was detected after **11.87h** compared to **13.79h** using BacT/ALERT™, resulting in an average advantage of **1.92h**. The table below outlines the classification metrics in hourly steps after detection using the CSA paradigm.

	Bal. Accuracy	Sensitivity	Specificity	PPV	NPV
At time of detection	94.71	90.70	98.72	86.67	99.14
time of detection +2h	96.65	93.33	99.96	99.56	99.31

Table 30: Time-resolved classification performance for *Serratia marcescens* [%]

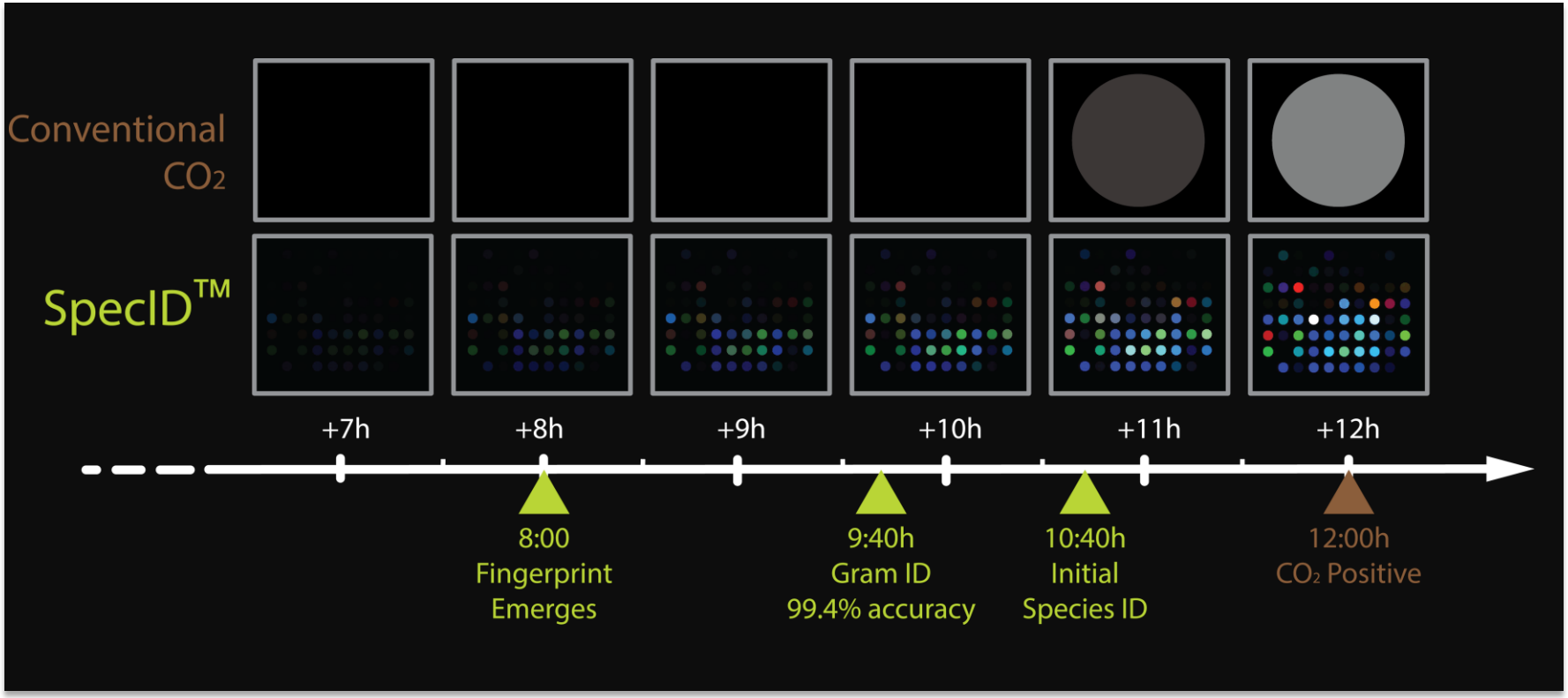

Figure 1: Time line of a bacterial growth experiment with *Klebsiella pneumoniae*. The top line visualizes the emergence of the BacT/ALERT™ fluorescence signal, the bottom line the CSA bacterial sensor fingerprint. Detection and identification events are annotated for both technologies and show the time advantage in detection and identification for the CSA system.

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