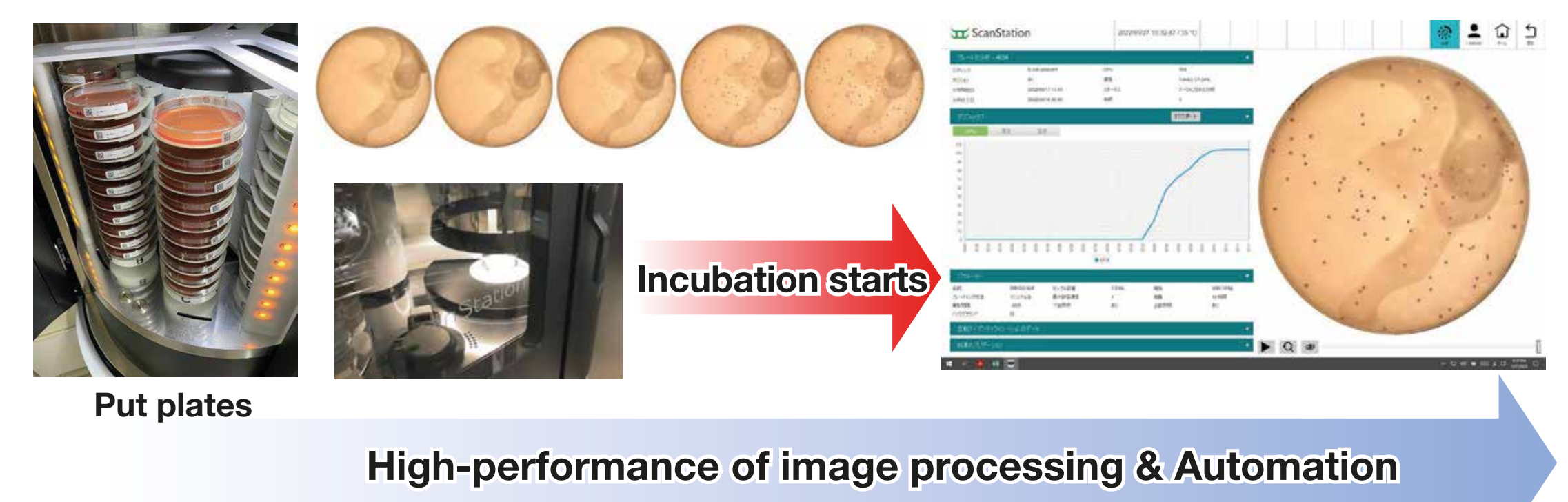


Investigation of further acceleration of result judgment by Rapid Media and real-time automatic colony counter

Kouki DATE, Tetuji TAKETOSHI, Yu ITO, Yoshiki HORI, Kouta OCHI, Misaki IWATA (Morinaga Milk Industry Co., Ltd. QC Department)

Objective

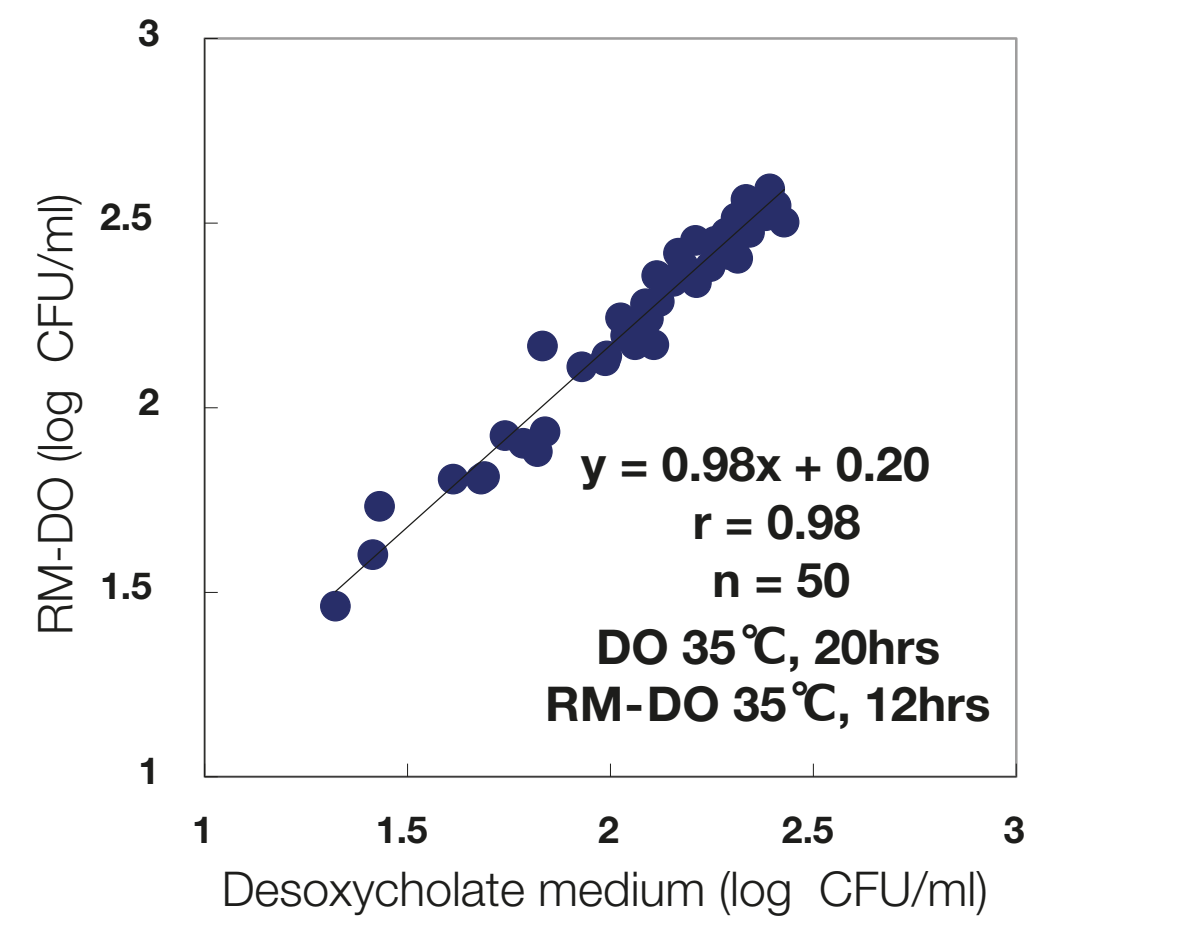
For food microbial inspection, faster results allow products to be shipped faster, especially for qualitative test, like coliforms, in case of a positive result. To act immediately with irregular results, it is important to get information faster. Desoxycholate medium (DO) which used for coliforms detection requires at least 18 hours. On the other hand, Rapid-media (RM-DO) requires only 12 hours to get the results, and this has been reported before [1]. Adding to this report, we report the possibility of further rapid detection of Escherichia coli with real-time & automatic colony counting system (ScanStation® by INTERSCIENCE made in France) which allows incubate and colony-counting at the same time during



Rapid Media-DO

● Rapid Media-DO (RM-DO) [1]

- **High-absorption capability**
Absorb 1mL sample within 30 minutes
- **High visibility**
Bacteria grows on the surface of plates. Compared to pour plates, we don't need to take agar's temperature effect into consideration.
- **X-GAL reagent method**
12 hours incubation equals to pour plate method (In case of pour & Desoxycholate (DO), it requires at least 18 hours)



● X-GAL reagent method

Simplification of confirmation test

Directly drop X-GAL reagent on the colonies of RM-DO medium, then we can simplify the confirmation of coliforms faster

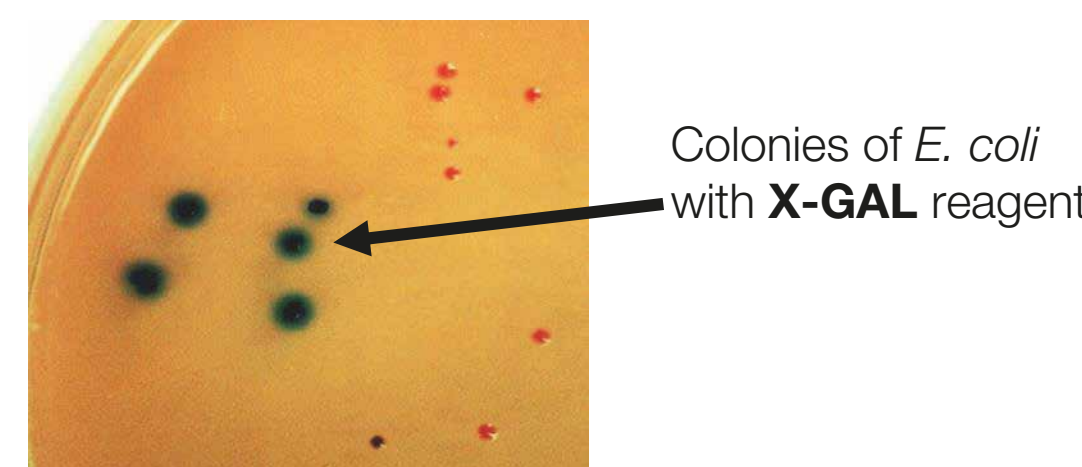
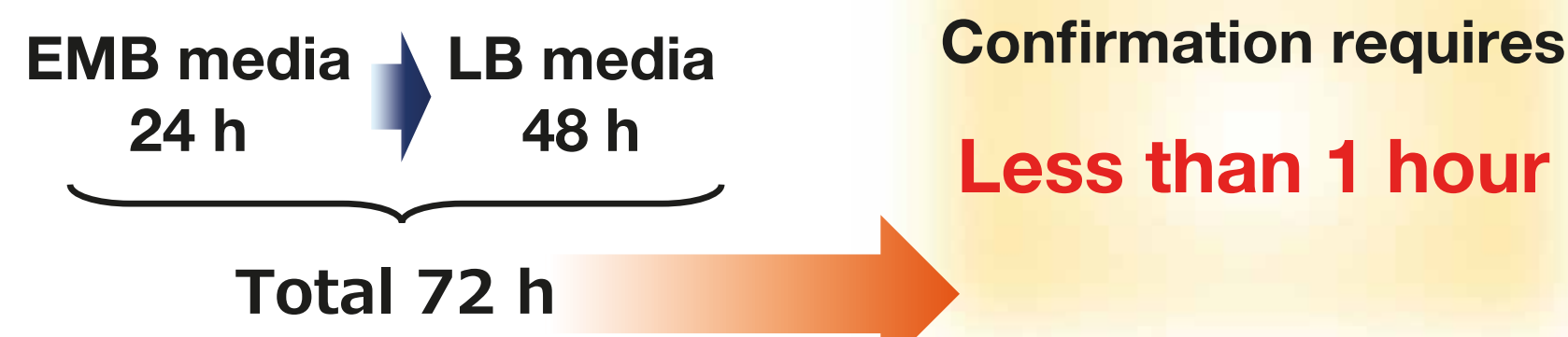
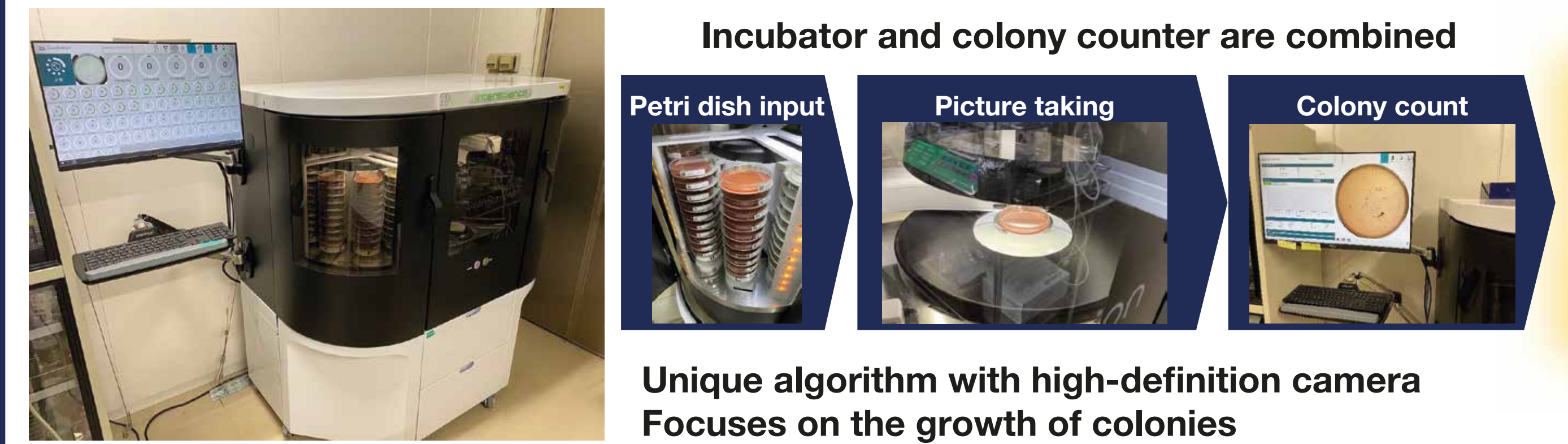


Fig. 2 Colonies of *E. coli* before & after X-GAL reagent

ScanStation®

● ScanStation®



Unique algorithm with high-definition camera
Focuses on the growth of colonies

ScanStation® monitors colonies on the plates every 30 minutes in 24 hours

Alert notification displayed once it detects a colony
Possible quick actions in case of emergency

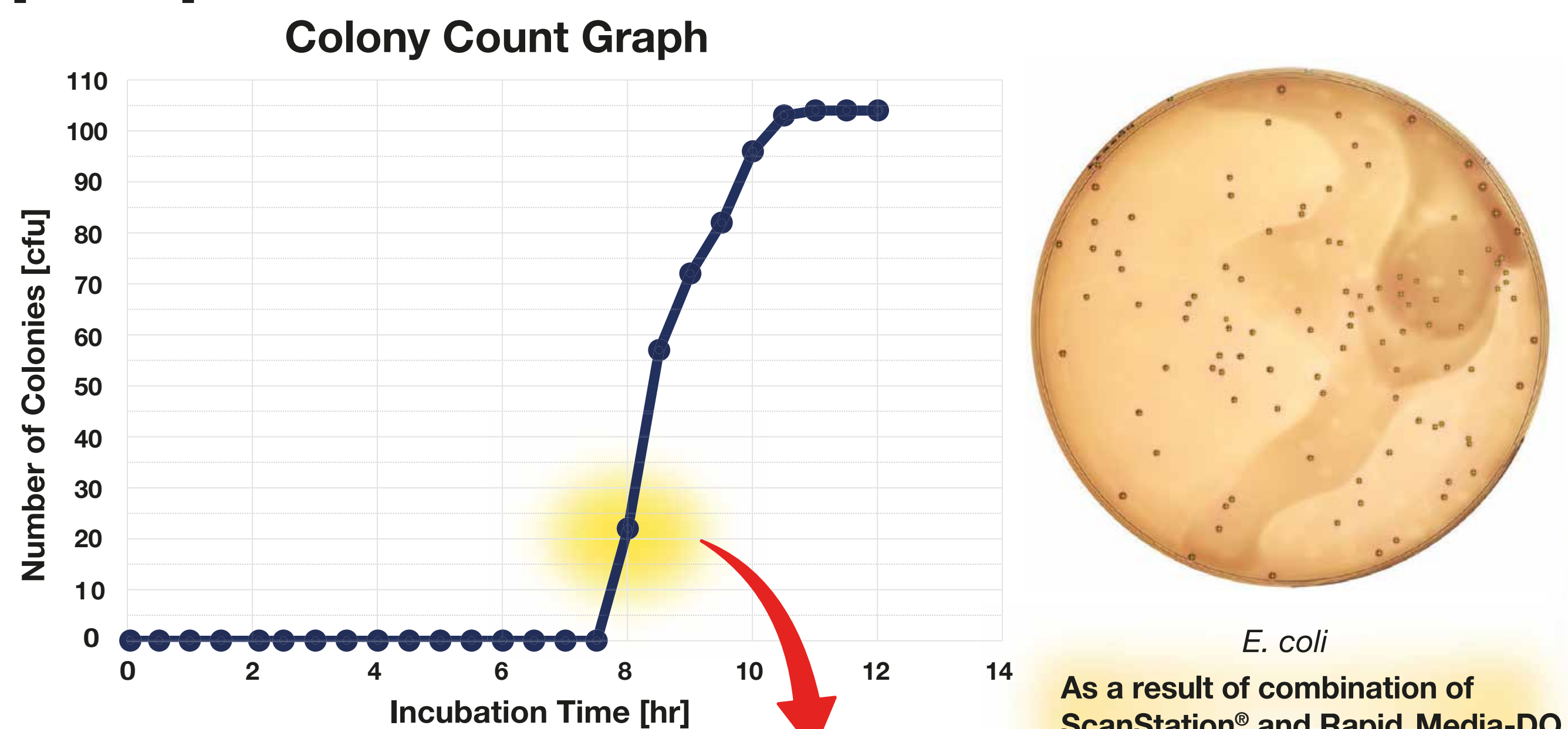
Rapidity of result judgment acquired with Rapid Media-DO & ScanStation®

● Verification of rapidity with Rapid Media-DO

[Method] Appx. 100 CFU / plate of *E. coli*

- ① Inoculate milk(100mL) with *E. coli* 10,000 CFU
- ② Plate 1.0mL of inoculated milk on RM-DO
- ③ Monitor the analysis of sample with ScanStation®

[Results]

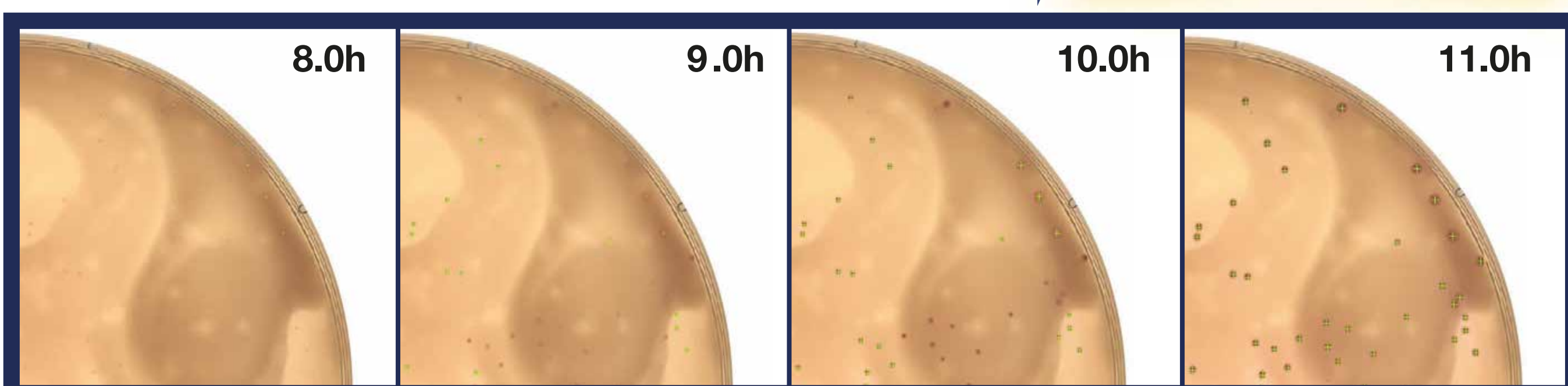


● It requires **only appx. 8 hours** to detect first colony.

Minimum detection time with Desoxycholate media ... 18 hours
Minimum detection time with Rapid-media(DO) 12 hours

As a result of combination of ScanStation® and Rapid Media-DO,

**We could reduce ;
10 hours maximum
4 hours maximum**



As result of 24h-monitoring and continuous image analysis, colonies are counted only when continuous growth is detected.

→ Possibility to earn information of colony-detection at early stage owing to high-definition images

Maximize the benefit of Rapid Media-DO with ScanStation® allows to detect colonies at early stage or very small colonies which is difficult for human eye to detect.

● Comparison between human eye and ScanStation®

Full-scale 8.0h Full-scale 12.0h

E. coli *E. coli*

The plates of which ScanStation® could detect colonies
The plates of which human eyes could recognize colonies

Impossible to count by human eyes We can observe; Neutral red & protuberance

Rapid Media-DO + ScanStation® = Rapidity of verification
Quick action at early stage is executable when we find abnormal results.

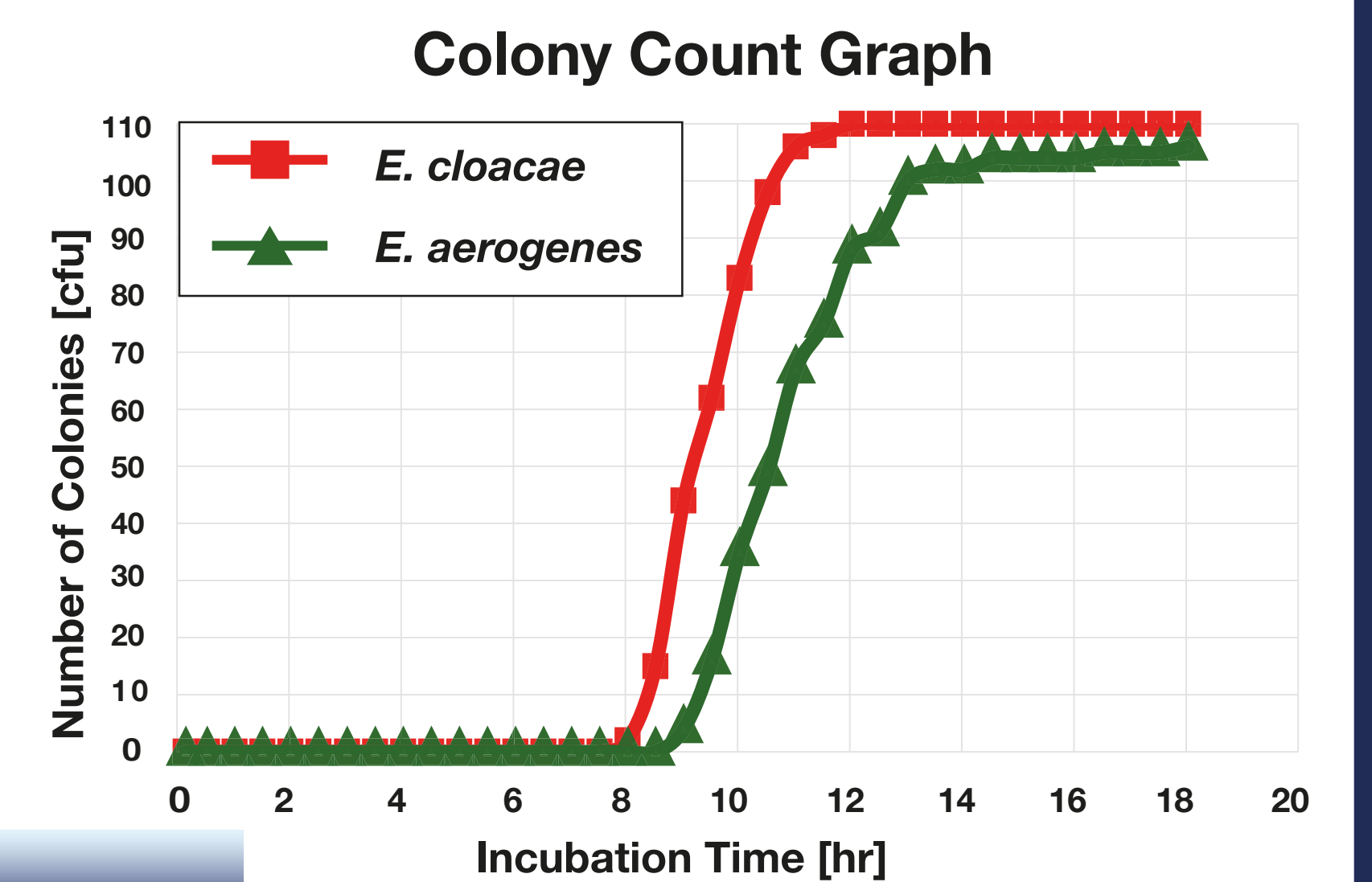
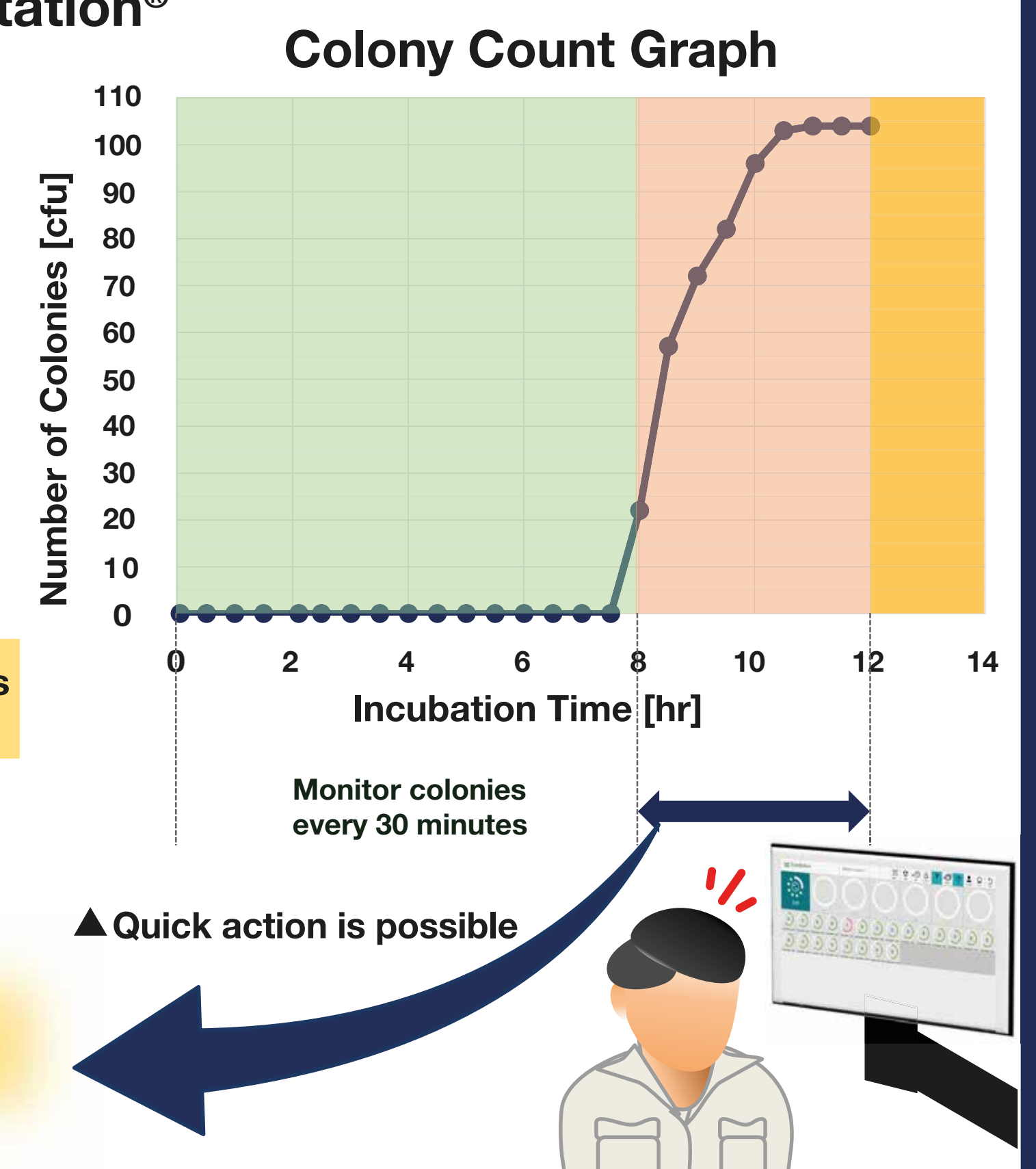
● Detection of *E. cloacae*, *E. aerogenes*

Analysis of difference between another species

E. cloacae *E. aerogenes*

The same as *E. coli*, ScanStation® could detect colonies earlier compared to Rapid Media-DO's 12-hours as detection time

Proves that ScanStation® could detect colonies at early stage regardless of types of species.



As a result of Rapid Media-DO & ScanStation®: action can quickly be taken when we face an issue

Conclusion

■ Use of Rapid Media-DO

→ Rapid detection of coliforms can be realized from **12 hours-incubation** at the earliest

■ Use of ScanStation®

Owing to 24h-monitoring and continuous image analysis, ScanStation® counts only colonies for which sufficient growth is found from successive images.
→ It makes possible to get accurate information of colony-detection at early stage.

With ScanStation®, it requires **appx. 8 hours at earliest** to detect **first small colony hard to see by human eyes.**

Combination of Rapid Media-DO and ScanStation® proves that we can take quick actions when we find abnormal results (appx. 8 hours at the earliest)