Evaluation of CD VP for fish and seafood by AOAC Validation Program

○Hajime Teramura, Shingo Mizuochi, Tadanobu Nirazuka, Masayuki Oikawa, Hidemasa Kodaka

(Nissui Pharmaceutical, Diagnostics Laboratory)

History or Purpose of Compact Dry VP Development

- Specification and standard for *V. parahaemolyticus* test on fish and seafood were fixed on June 7, 2001.

- Food poisoning by *V. parahaemolyticus* occurs every year even though the occurrence cases decrease.

- In order to decrease food poisoning by *V. parahaemolyticus*, Compact Dry VP was developed as simple and convenient medium for customers.

Evaluation of Compact Dry VP

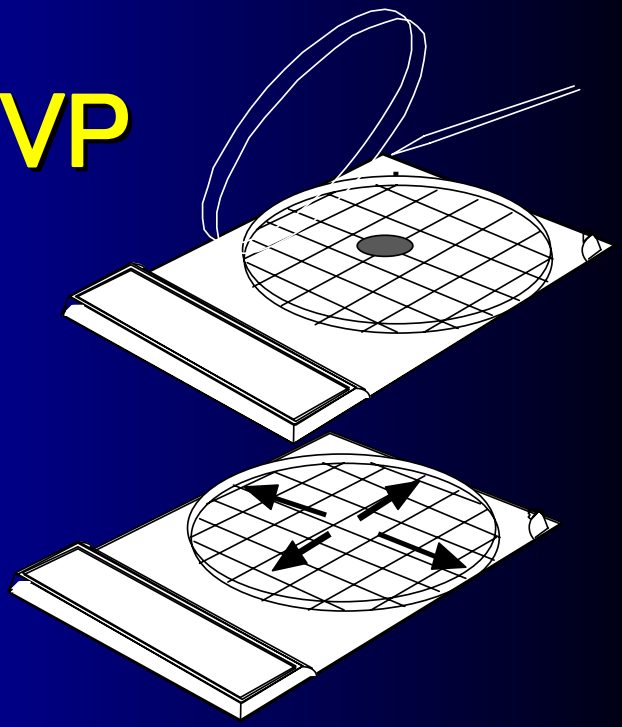
Test fish and seafood according to Validation Program by AOAC Research Institute.

Method	Fresh fish and seafood	Processed fish and seafood
CDVP	1ml inoculation	1ml inoculation
FDA BAM *	Colony hybridization	MPN (Most Probable Number)

* Bacteriological Analytical Manual online (January 2001)

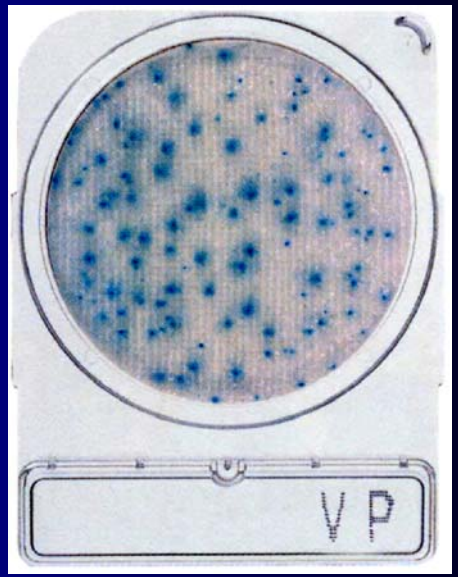
Outline of Compact Dry VP

Simple and convenient medium for *V. parahaemolyticus* with sterilized device and no-need of medium preparation



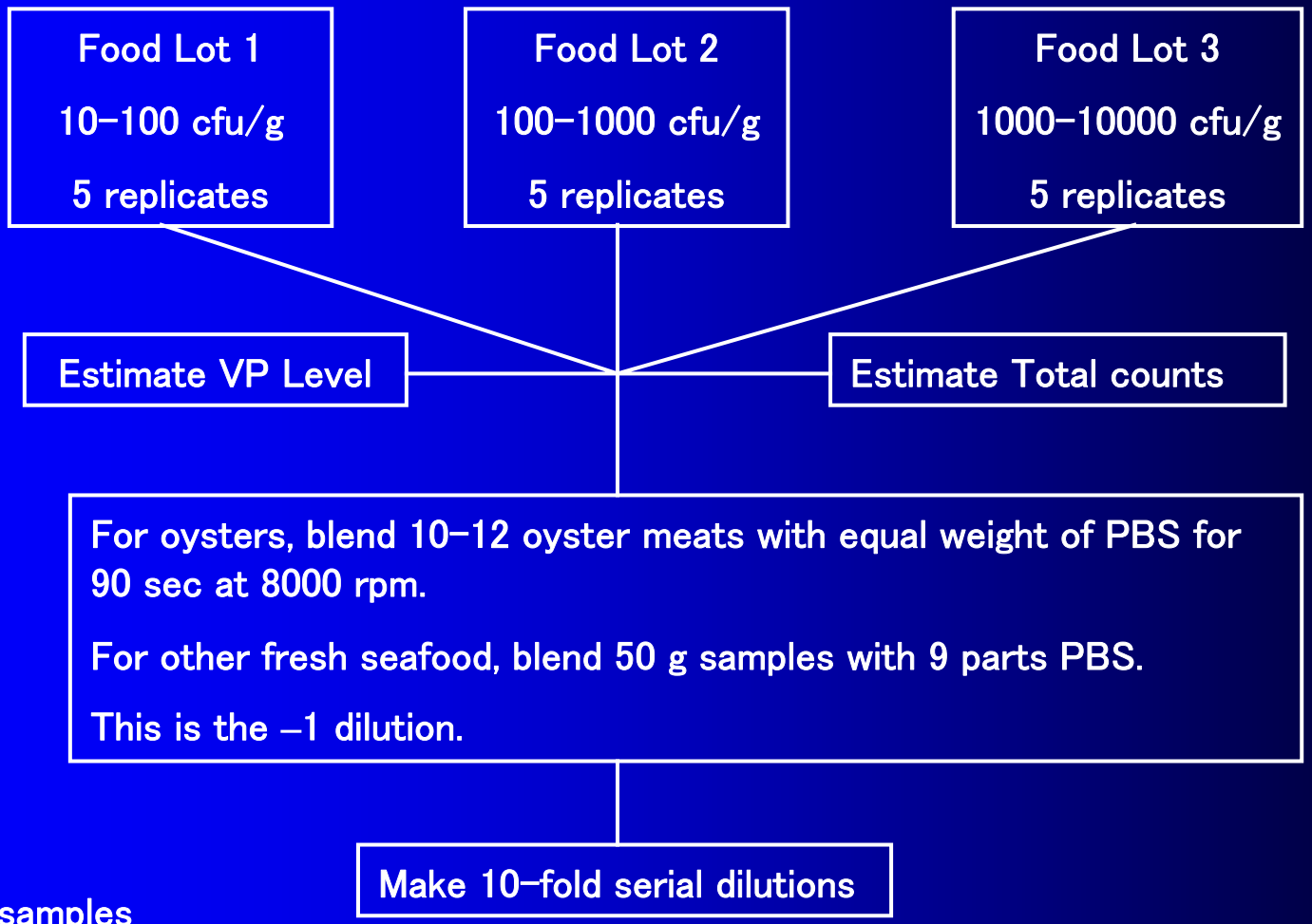
No need of smearing procedure since sample diffuses automatically soon after dropping sample on plate

V.parahaemolyticus generate blue colony by enzyme chromogenic substrate



Drop 1ml sample on plate, incubate the plate at 36±1℃ for 19±1 hours, and count

Sample Preparation



※Food samples

Fresh Seafood：fresh raw oysters (Vp ATCC 27969)、fresh raw tuna (Vp RIMD 2212197)

Processed Seafood：frozen raw scallops (Vp NS7160)、frozen raw salmon (Vp RIMD 2210371)

FDA BAM Method, Colony hybridization Method for Fresh Fish and Seafood

Oysters: spread plate 0.2g of the –1 dilution and 100µl of –2 and –3 dilutions onto T1N3 (g/L: Trypton 10, NaCl 30, Agar 20, pH7.2).

Other seafood: Spread plate 100µl of –1, –2 and –3 dilutions onto T1N3, incubate 18–24h@35℃.

↓

Overlay T1N3 plate with labeled filters (whatman #541) for 1–30 min.

↓

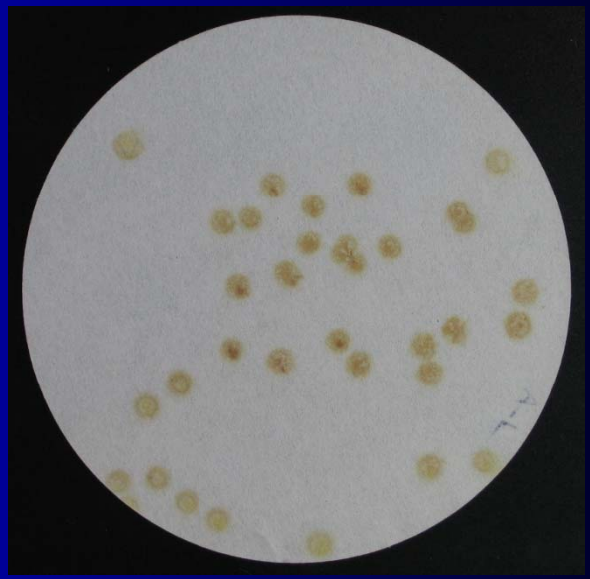
Lyse, neutralize, rinse, treat with Proteinase K and rinse according to protocol.

↓

Probe with AP–labeled *tlh* the probe (DNA Technology A/S) on the filters @54℃.

↓

Develop filters with NBT/BCIP. Count purple or brown spots.



FDA BAM Method, MPN Method for Processed Fish and Seafood

Inoculate 3-tube MPN in APW [1] ; incubate overnight@35℃.

↓

Streak to TCBS; incubate overnight@35℃.

↓

V.parahaemolyticus appear as round, opaque, green or bluish colonies, 2-3 mm in diameter.

↓

Confirm according to BAM using traditional biochemicals [2] , API20E, or probe hybridization

↓

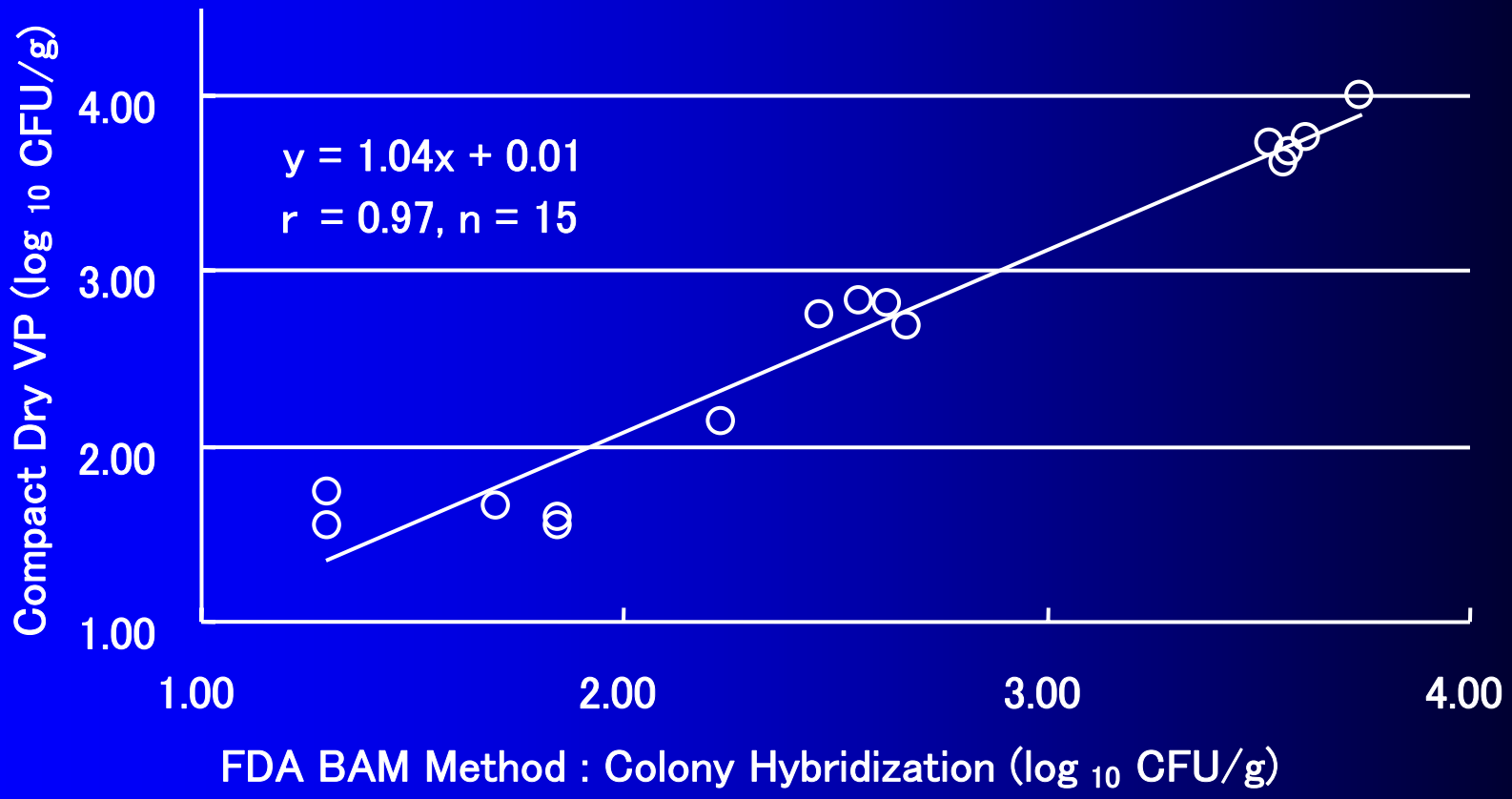
Determine MPN

1) APW ; Alkaline Peptone Water

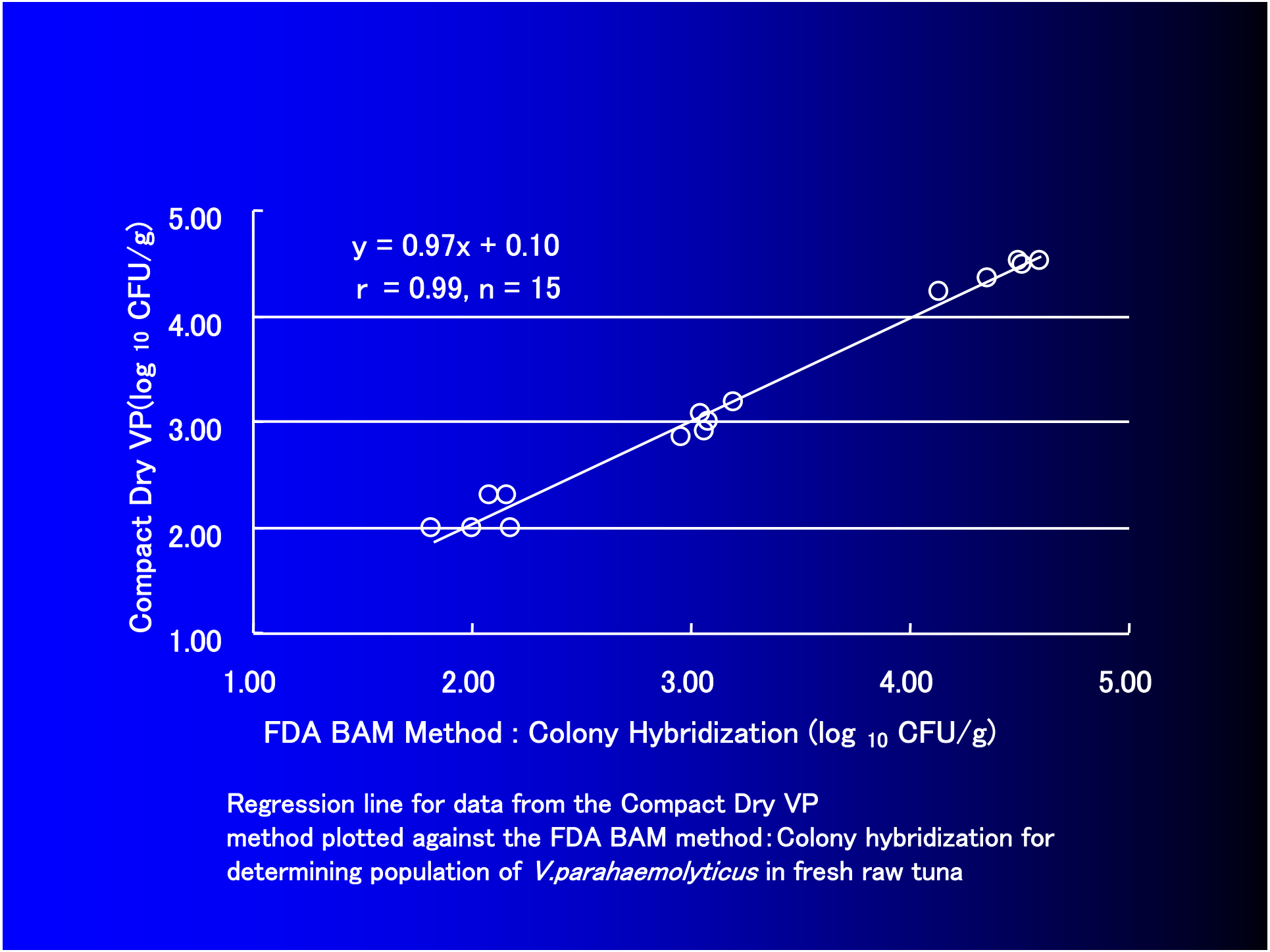
(g/L : peptone 10, NaCl 10, pH 8.5±0.2)

2) Traditional biochemicals

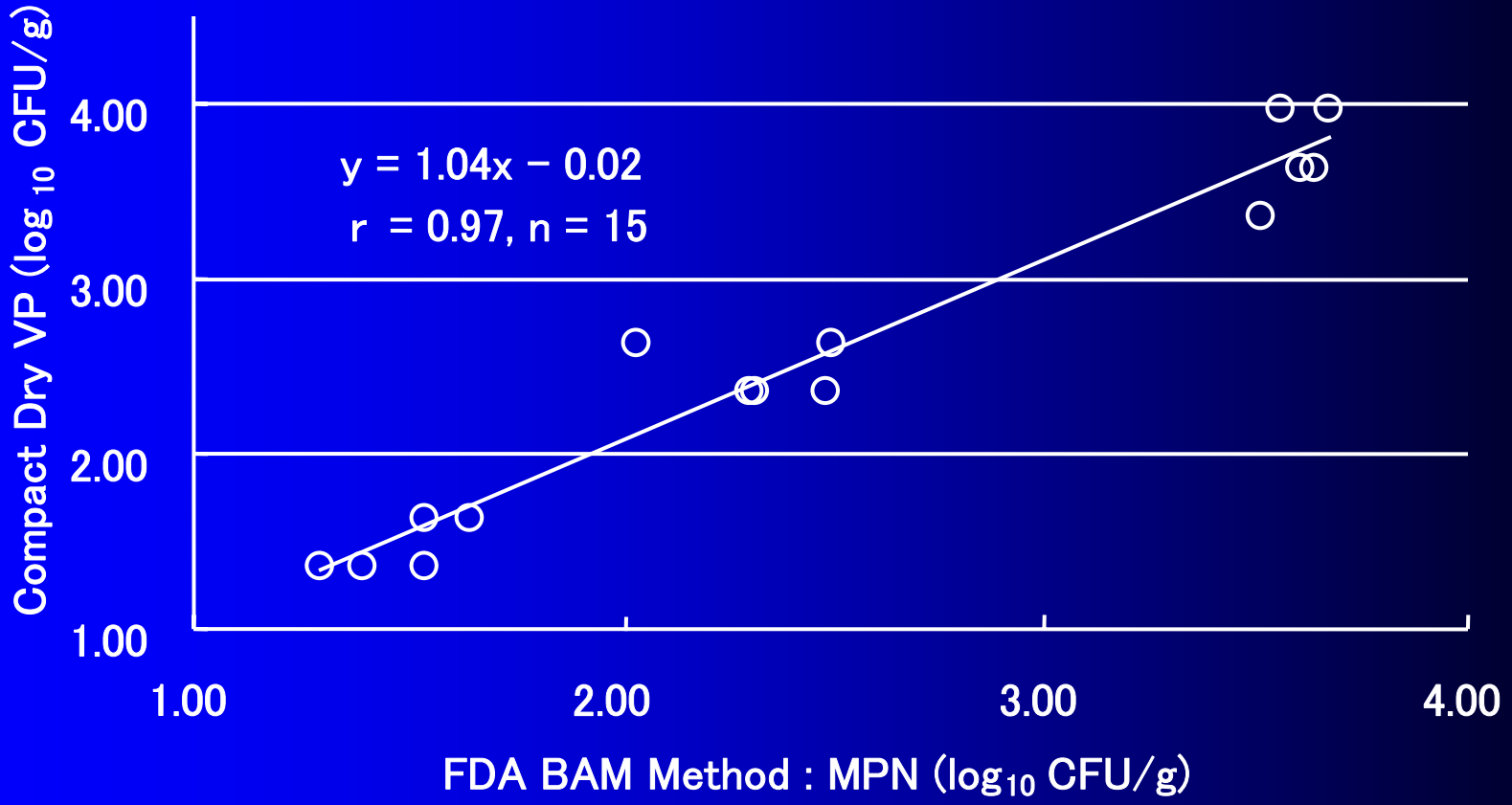
Gram stain、oxydase test、Arginine glucose slant (AGS)、TSI (contain 2% NaCl)、Motility test、ONPG tset、salt-tolerance、O/129 sensitivity、API20E



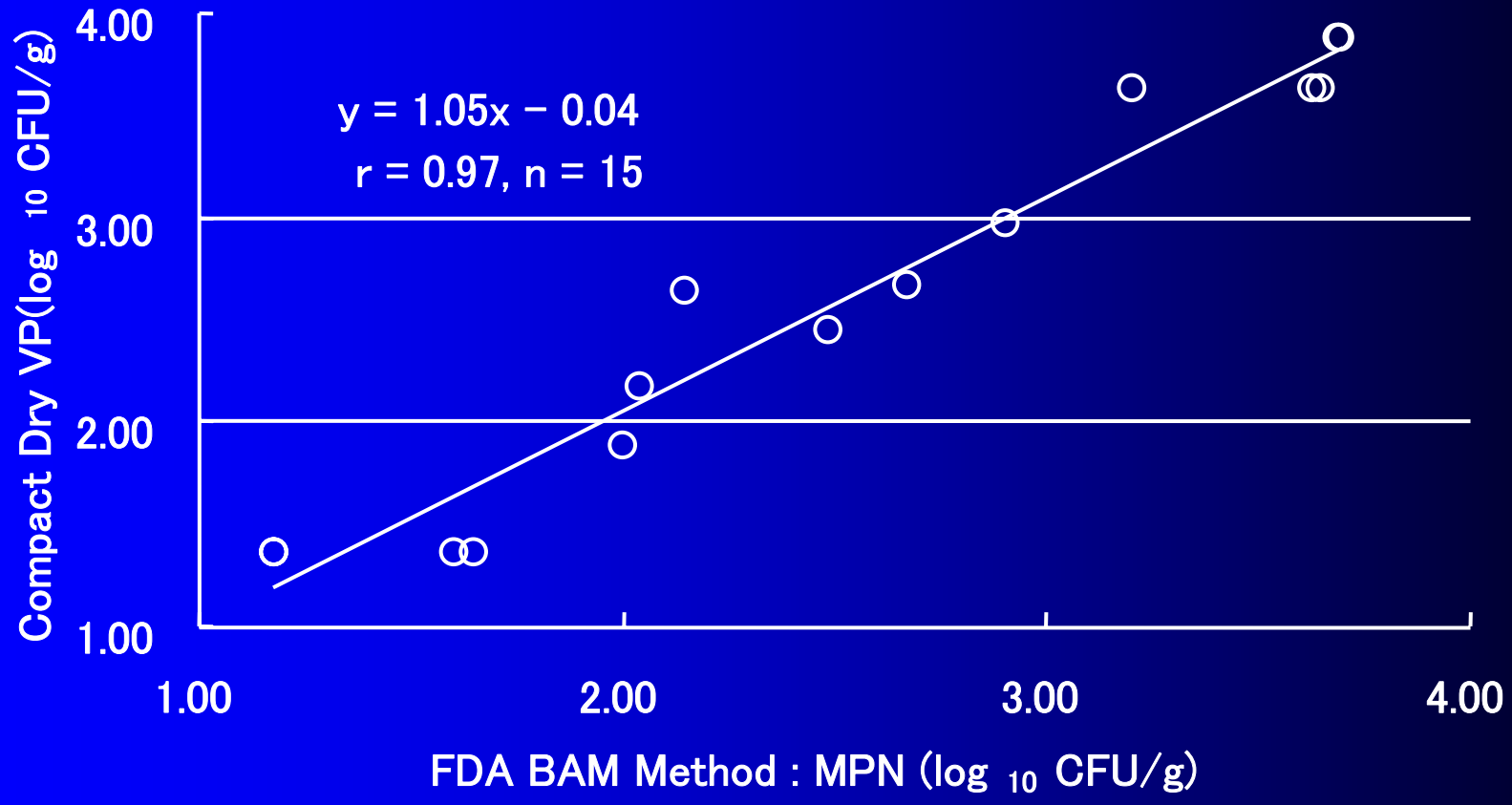
Regression line for data from the Compact Dry VP method plotted against the FDA BAM method : Colony hybridization for determining population of *V.parahaemolyticus* in fresh raw oyster



Regression line for data from the Compact Dry VP method plotted against the FDA BAM method : Colony hybridization for determining population of *V.parahaemolyticus* in fresh raw tuna



Regression line for data from the Compact Dry VP method plotted against the FDA BAM method : MPN for determining population of *V.parahaemolyticus* in frozen scallops



Regression line for data from the Compact Dry VP method plotted against the FDA BAM method : MPN for determining population of *V.parahaemolyticus* in frozen salmon

Evaluation result by AOAC Validation Program

- Correlation between CD VP and FDA BAM (Colony Hybridization) for fresh raw oyster and tuna shows no significant difference ($P>0.05$). And it indicates good correlation with correlation coefficient of $r=0.97$、$r=0.99$ and regression line of $Y=1.04x + 0.01$、$Y=0.97x + 0.10$.

- Correlation between CD VP and FDA BAM(MPN method) for frozen scallop and salmon shows no significant difference ($P>0.05$). And it indicates good correlation with correlation coefficient of $r=0.97$、$r=0.99$ and regression line of $Y=1.04x - 0.02$、$Y=1.05x - 0.04$.

Summary

Correlation between CD VP and FDA BAM (colony hybridization or MPN method) for *V. parahaemolyticus* on fish and seafood shows good relationship and no significant difference. And procedure of CD VP is more convenient and easier judgment than FDA BAM, and therefore we believe CD VP is useful tool to detect *V. parahaemolyticus* on fields of seafood processing and food distribution industry.