The study results on previous and new extraction methods for Loopamp® *Salmonella* Detection Kit

Since the release of Loopamp® *Salmonella* Detection Kit in March 2003, it has been adopted for food inspection by many testing centers, food manufacturers etc. However, samples with high lipid content such as poultry or compressed-oil residuals, might inhibit LAMP reactions and show negative results even though culture methods show positive results.

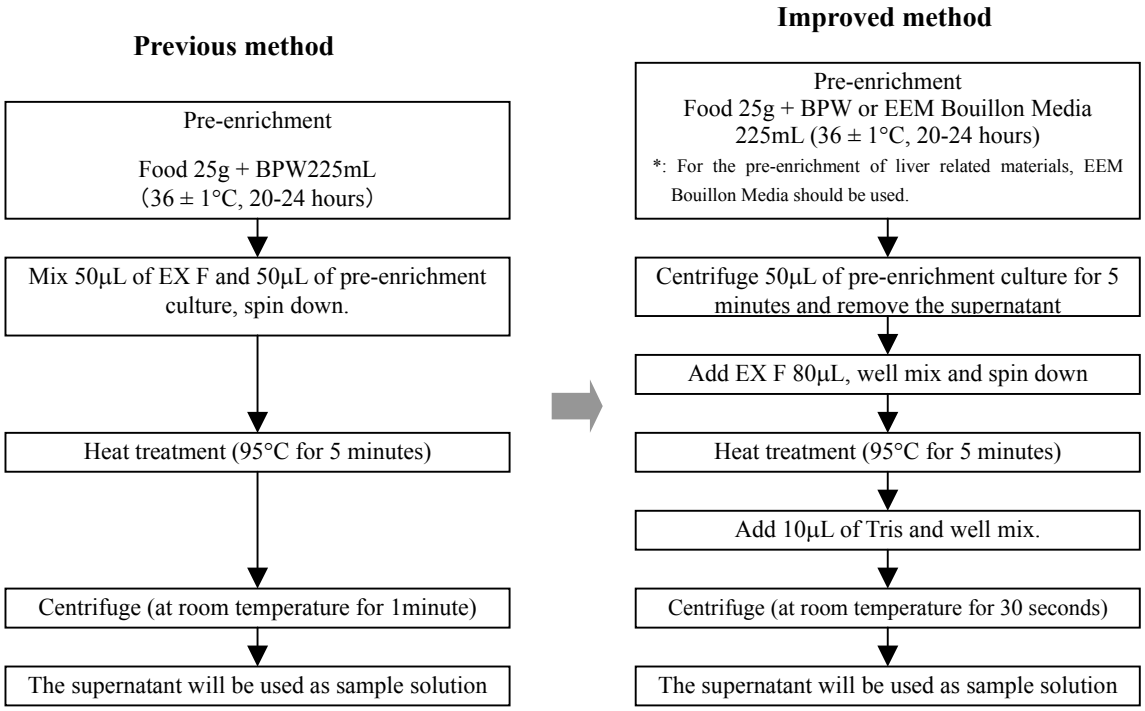
Eiken Chemical Co., Ltd. has been researching on improving the extraction method. We here would like to introduce a solution that not only better solve the inhibition problem on high lipid samples, but also shorten the reaction time for the detectable samples by previous extraction method (hereinafter referred to as previous method), and show a more prominent turbidity increase of magnesium pyrophosphate precipitate. We adopt this method as the new extraction method (hereinafter referred to as improved method). Previous method was known to have interference from EEM Bouillon Media, but this interference has been avoided in improved method.

As a result, improved method has been adopted for handling all the samples, and the contents of the kit, operation procedures and package insert has been modified accordingly to reflect the changes. The study results on the previous and new extraction methods using different samples and enrichment media are summarized as follows.

1. The operation procedures of improved method

Apart from the procedures of previous method, improved method contains the procedures such as centrifuge and removing supernatant, neutralization after heat treatment etc.

First, dispense 50μL of the pre-enrichment culture into each tube as samples, and centrifuge for 5 minutes. Then remove the supernatant carefully as to not convolute the precipitate. If there is no precipitate in the solution, remove 40μL of supernatant. Add 80μL of Extraction Solution for Foods (EX F), well mix and spin down for a few seconds, and then incubate at 95°C for 5 minutes. Add 10μL of 1M Tris-HCl: pH7.0 (Tris) and well mix, centrifuge for 30 seconds at room temperature. The supernatant will be used as sample solution (Figure below. Refer to the package insert for more details).



2. Study on extraction methods

[Method of study]

Mix 25g of sample with 225g of liquid enrichment media for Salmonella (Buffered Peptone Water (hereinafter

referred to as BPW) or EEM Bouillon Media (hereinafter referred to as EEM)). After stomachering, incubate at 37°C for 24 hours (in some cases 16 hours) for enrichment. And then prepare the stimulated samples to contain 2.4 X 10^5 CFU/mL or 2.4 X 10^4 CFU/mL of *Salmonella* Enteritidis.

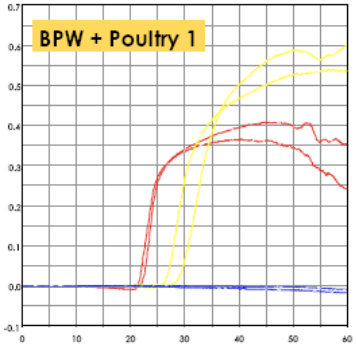
Use previous method and improved method respectively to conduct the extraction from the simulated samples, and take 5μL (600 CFU/test or 60 CFU/test) each for the LAMP reactions.

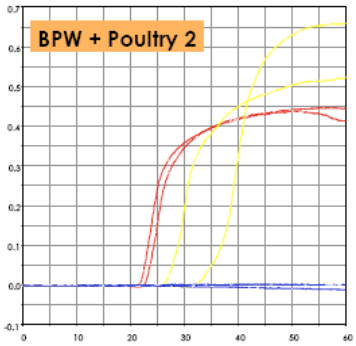
The results of LAMP reactions using different food materials and enrichment media are summarized as follows.

1) The study on poultry samples

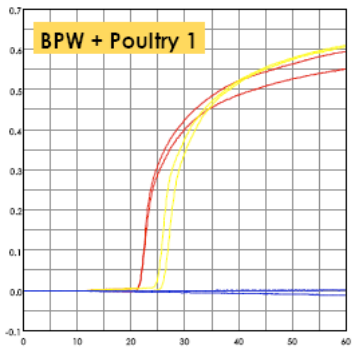
With the previous method, lipid content from poultry might interfere LAMP reactions. Samples were cultured by BPW Media in this study, although previous method showed inhibition in turbidity increase, improved method showed a clear turbidity increase pattern.

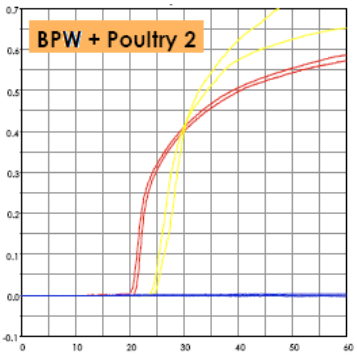
<Previous method>





<Improved method>





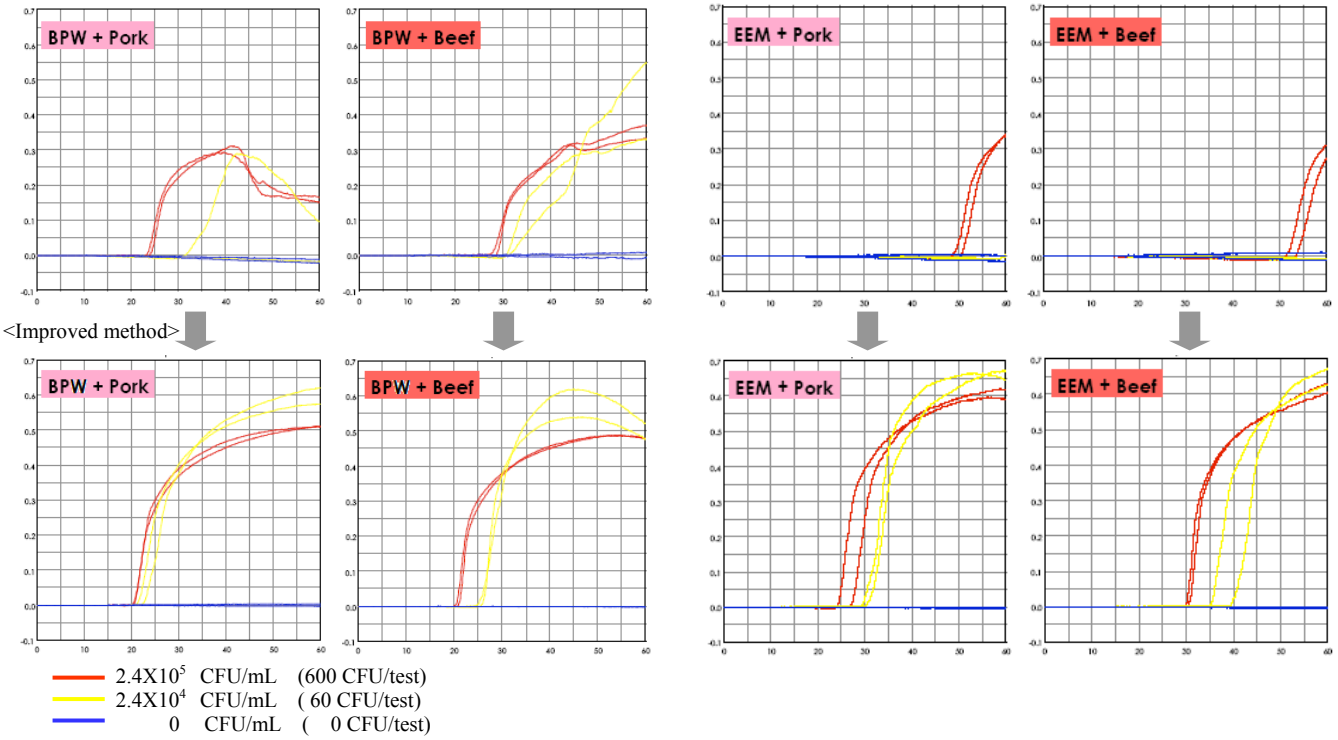
2.4X10^5 CFU/mL (600 CFU/test)
2.4X10^4 CFU/mL (60 CFU/test)
0 CFU/mL (0 CFU/test)

2) The study on pork, beef and beef liver samples

When meat samples were cultured by BPW Media, although previous method showed interference in both pork and beef samples, improved method showed a clear turbidity pattern and the amplification starting time could also be observed earlier. When cultured by EEM Media, previous method has shown more severe interference than its BPW results. In contrast, when improved method was used, although the starting time of amplification is slower than the ones using BPW Media, its amplification was recognized much faster than the previous method and the amplification could be clearly confirmed.

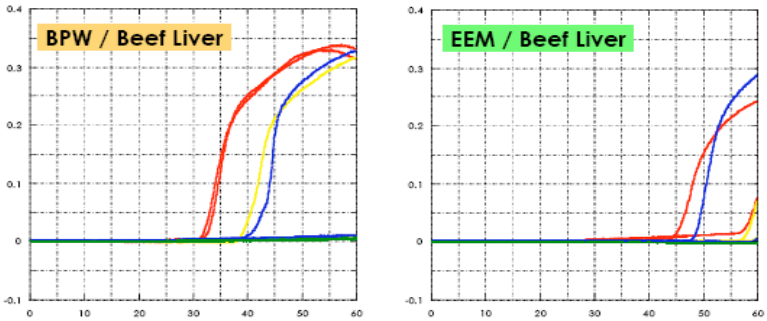
<Previous method>

<Improved method>

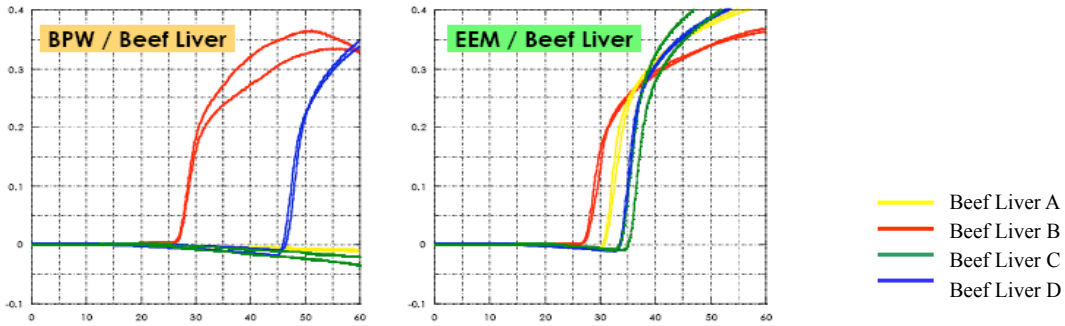


In the study of beef liver, pre-enrichment were conducted with 4 samples (sample A, B, C, D) for 16 hours, and then mixed with the bacteria solution (2.4 X10^5CFU/mL). When BPW Media were used, inhibition to reaction was observed in previous method, and in some cases, amplification could not be recognized within 60 minutes. When BPW Media were used with improved method, some samples even showed more severe inhibition. However, when EEM was used with improved method, although previous method showed more significant inhibition in EEM than BPW Media, improved method did not have any problem in all the detections. Therefore, EEM Media should be used for the pre-enrichment of beef liver samples.

<Previous method>



<Improved method>

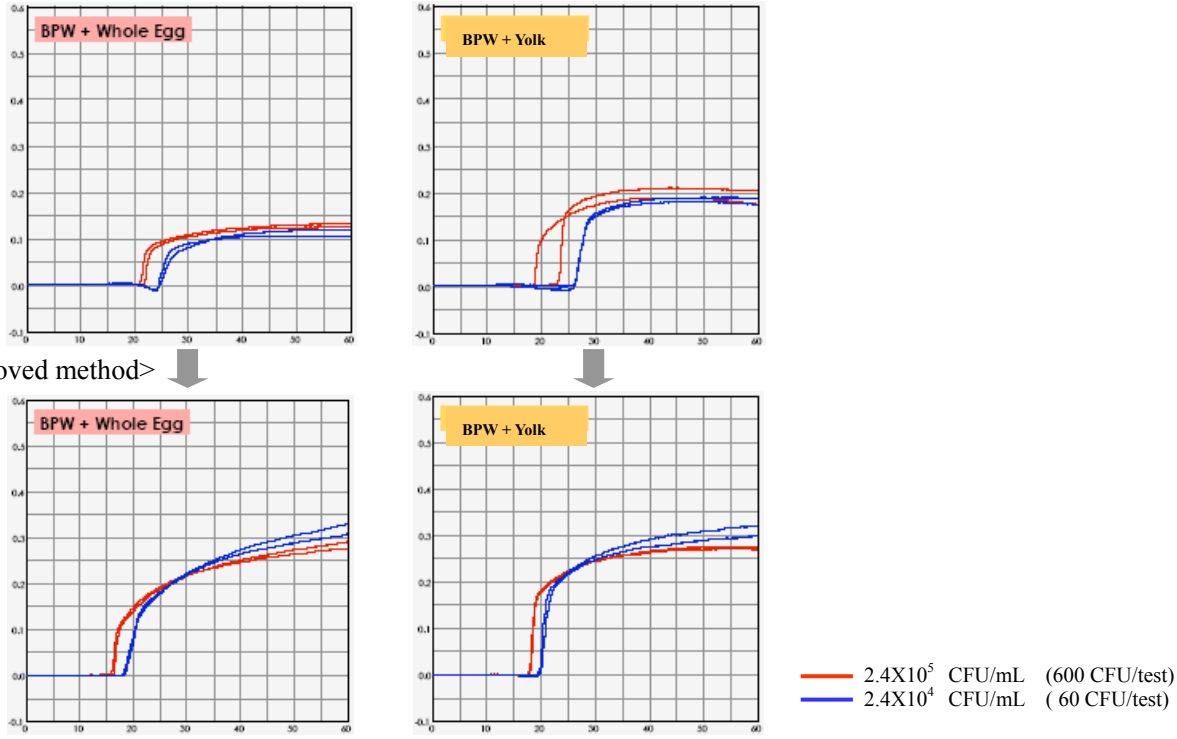


3) The study on egg samples

Whole egg and yolk only were cultured in BPW Media. Although amplification could be observed in previous method, turbidity increase was significantly inhibited. When improved method was used, not only turbidity increase became more prominent, the amplification starting time could also be observed earlier. It seems that the inhibition elements to LAMP reactions in the enrichment culture had been removed by centrifuge operation.

<Previous method>

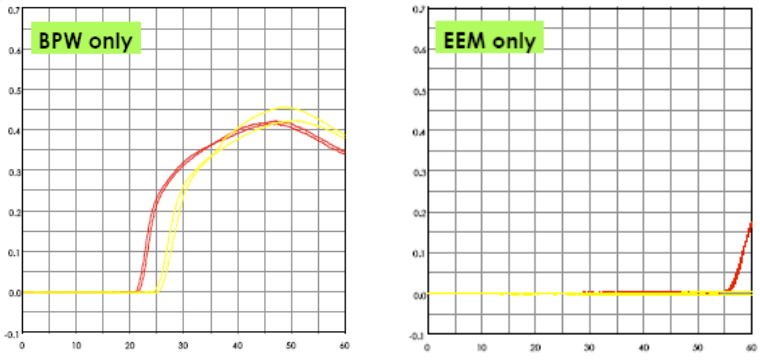
<Improved method>



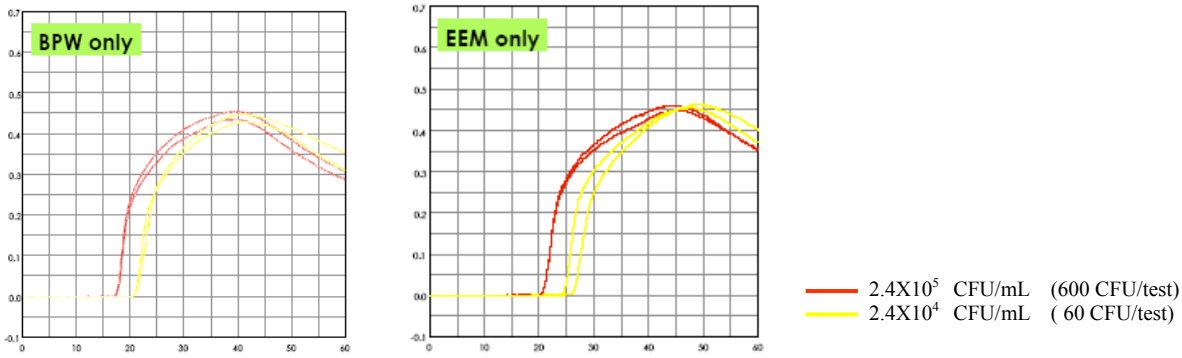
4) The study on using only enrichment media (BPW or EEM)

Incubated only the enrichment media at 37°C for 24 hours and added bacteria into them. For BPW Media with improved method, the amplification starting time could be observed earlier than previous method. When EEM Media was used, although previous method did not show amplification at 60CFU/test within 60 minutes, improved method showed the turbidity increase after about 25 minutes of the amplification.

<Improved method>



<Improved method>



5) The study on compressed-oil residual samples

In the study of compressed-oil residuals, cultured bacteria were added into the BPW Media. For the compressed-oil residuals of wheat germ, while previous method showed some inhibition, improved method showed better turbidity increase. Improved method also showed more prominent amplification curves and the amplification starting time could be observed earlier. For the compressed-oil residuals of coleseed, although previous method did not show any turbidity increase, improved method showed the turbidity increase after about 20 minutes of the amplification.

<Previous method>

<Improved method>