

Global approach of the uncertainty of measurement in microbiology

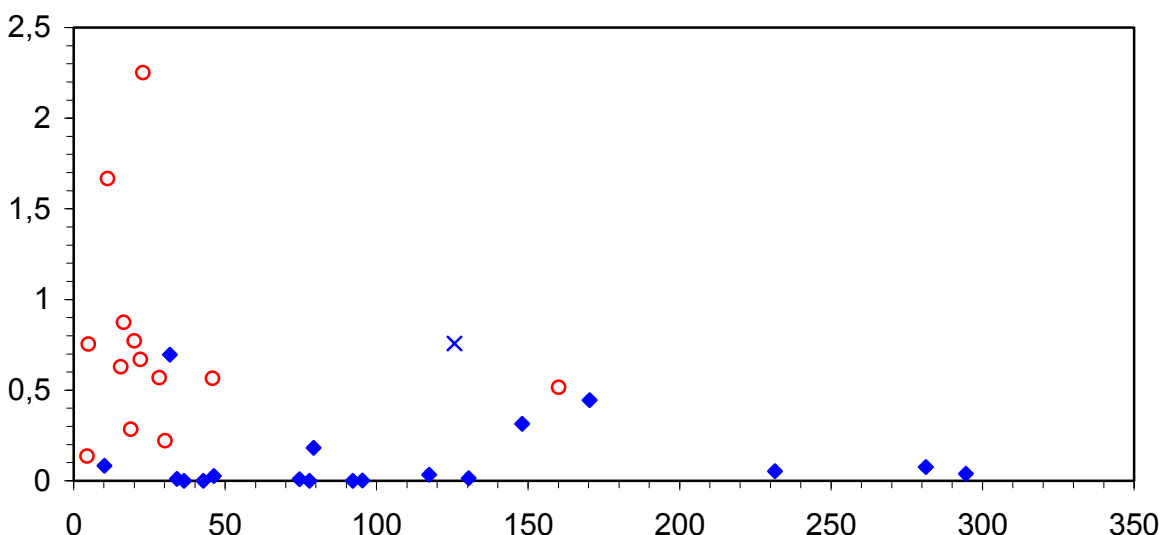
Interlaboratory trials organised by *AGLAE* between 1994 and 2005

Philippe Guarini, Olivier Molinier, Karine Vidor
January 18, 2006

Enumeration of colony counts ('culturable micro-organisms') at 22°C in 1 ml

Test materials:

◆ Artificially contaminated waters ('spikes') ○ BioReference pastilles × Naturally contaminated samples



Each data point represents the result of an interlaboratory trial.

x-axis: mean number of colony count in 1 ml (arithmetic mean).

y-axis: squared coefficient of variation of the method ($CV_{\text{meth}}^2 = CV_R^2 - CV_{\text{Poisson}}^2$).

Notes:

1/ One trial is not represented due to anomalies detected in the quality of the test material sent to participants; comparative trial 03M30.2.

2/ Several trials are not represented on the figure: the ones where the target organisms were not present in the test material and some trials with artificially contaminated waters for which the dispersion was limited to a Poisson distribution with a microbial load inferior to 10 in average per test portion volume.

Using artificially contaminated waters:	$CV_{\text{meth}}^2 =$	0,110	
Using naturally contaminated samples:	$CV_{\text{meth}}^2 =$	0,757	(one single trial)
Using BioReference pastilles:	$CV_{\text{meth}}^2 =$	0,762	

Expression of the uncertainty of measurement

Regulation value (in France) = 100 colony counts at 22°C in the 1 ml analysed;

therefore, expression of the uncertainty of measurement in terms of confidence interval around the regulation value (CI₁₀₀).

CI₁₀₀ = [50 ; 300] colony counts at 22°C in average in 1 ml if the germs are not stressed.
(note: CI₁₀₀ with natural variation only is [80 ; 120])

CI₁₀₀ = + than 50 colony counts at 22°C in average in 1 ml if the germs are stressed.
(note: value obtained with BioReference pastilles)

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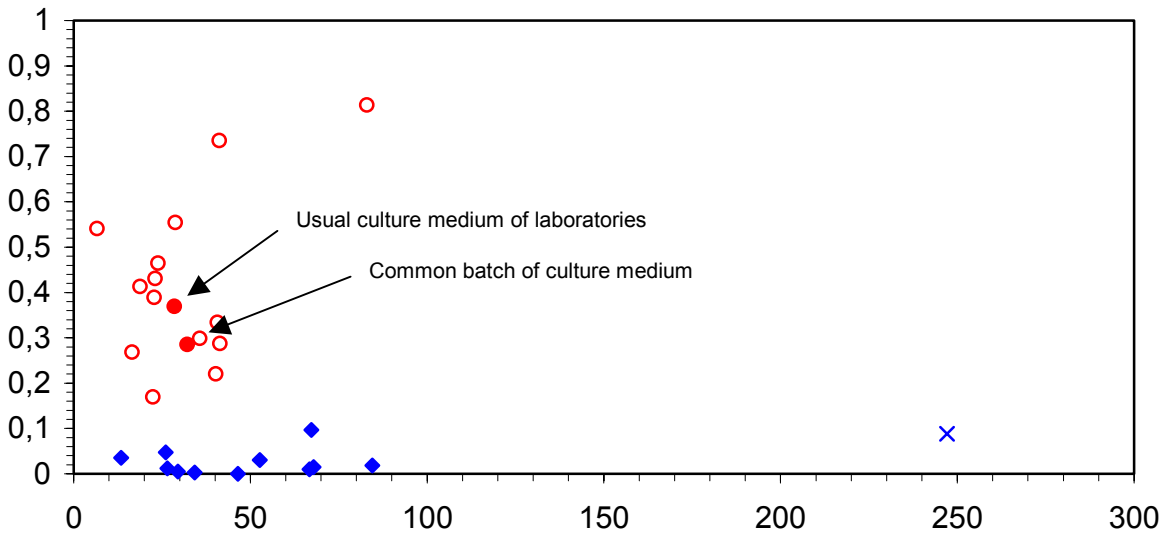
Interlaboratory trials organised by *AGLAE* between 1994 and 2005

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January 18, 2006

Enumeration of total coliforms in 100 ml

Test materials:

- ◆ Artificially contaminated waters ('spikes')
- BioReference pastilles
- × Naturally contaminated samples
- Trial 03M30.2 on BioReference pastilles



Each data point represents the result of an interlaboratory trial.

x-axis: mean number of total coliforms in 100 ml (arithmetic mean).

y-axis: squared coefficient of variation of the method ($CV_{\text{meth}}^2 = CV_R^2 - CV_{\text{Poisson}}^2$).

Notes:

- 1/ Two trials are not represented due to anomalies detected in the quality of the test material sent to participants.
- 2/ Several trials are not represented on the figure: the ones where the target organisms were not present in the test material and some trials with artificially contaminated waters for which the dispersion was limited to a Poisson distribution with a microbial load inferior to 10 in average per test portion volume.
- 3/ Trial 03M30.2 was a comparative trial aimed at quantifying the effect of the 'supplier of culture media' factor; the participants performed analyses on their culture medium and on a common medium (unique batch sent out for the trial).

Using artificially contaminated waters:	$CV_{\text{meth}}^2 =$	0,025	
Using naturally contaminated samples:	$CV_{\text{meth}}^2 =$	0,088	(one single trial)
Using BioReference pastilles:	$CV_{\text{meth}}^2 =$	0,364	

Expression of the uncertainty of measurement

Regulation value (in France) = absence of total coliforms in the 100 ml analysed;

therefore, expression of the uncertainty of measurement in terms of limit of detection (LD).

LD = 3,1 total coliforms in average in 100 ml if the germs are not stressed.

(note: LD with natural variation only is 3,0)

LD = 5,4 total coliforms in average in 100 ml if the germs are stressed.

(note: value obtained with BioReference pastilles)

Caution: the fact not to pick all the typical colonies for confirmation tests leads to higher LD.

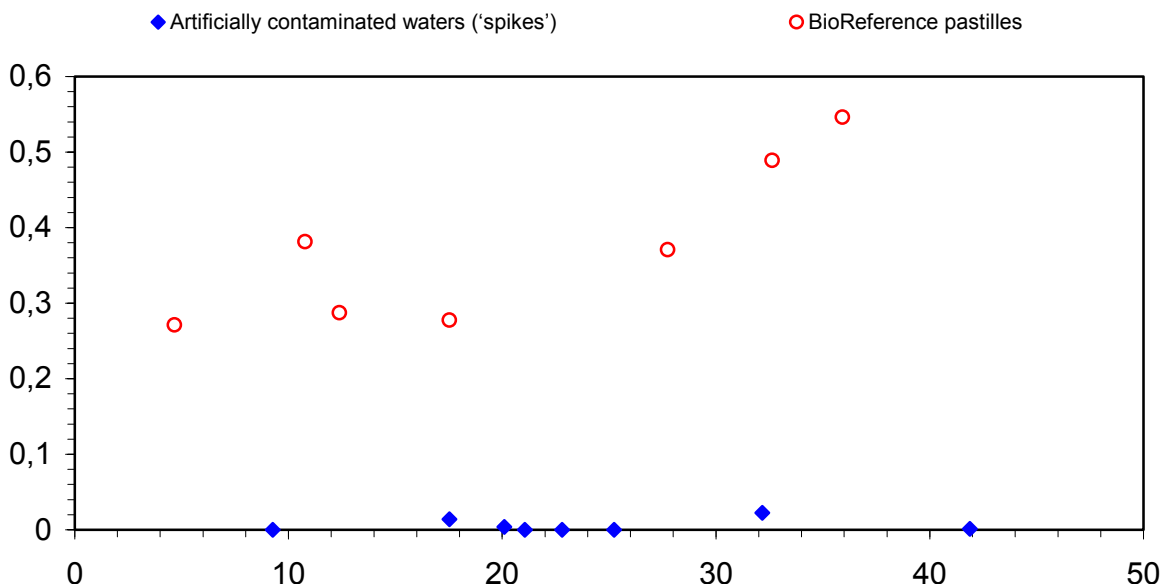
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Interlaboratory trials organised by *AGLAE* between 1994 and 2005

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Enumeration of thermotolerant coliforms in 100 ml

Test materials:



Each data point represents the result of an interlaboratory trial.

x-axis: mean number of thermotolerant coliforms in 100 ml (arithmetic mean).

y-axis: squared coefficient of variation of the method ($CV_{\text{meth}}^2 = CV_R^2 - CV_{\text{Poisson}}^2$).

Notes:

1/ Several trials are not represented on the figure: the ones where the target organisms were not present in the test material and some trials with artificially contaminated waters for which the dispersion was limited to a Poisson distribution with a microbial load inferior to 10 in average per test portion volume.

Using artificially contaminated waters: $CV_{\text{meth}}^2 = 0,005$

Using BioReference pastilles: $CV_{\text{meth}}^2 = 0,375$

Expression of the uncertainty of measurement

Regulation value (in France) = absence of thermotolerant coliforms in the 100 ml analysed; therefore, expression of the uncertainty of measurement in terms of limit of detection (LD).

LD = 3,0 thermotolerant coliforms in average in 100 ml if the germs are not stressed.
(note: LD with natural variation only is 3,0)

LD = 5,5 thermotolerant coliforms in average in 100 ml if the germs are stressed.
(note: value obtained with BioReference pastilles)

Caution: the fact not to pick all the typical colonies for confirmation tests leads to higher LD.

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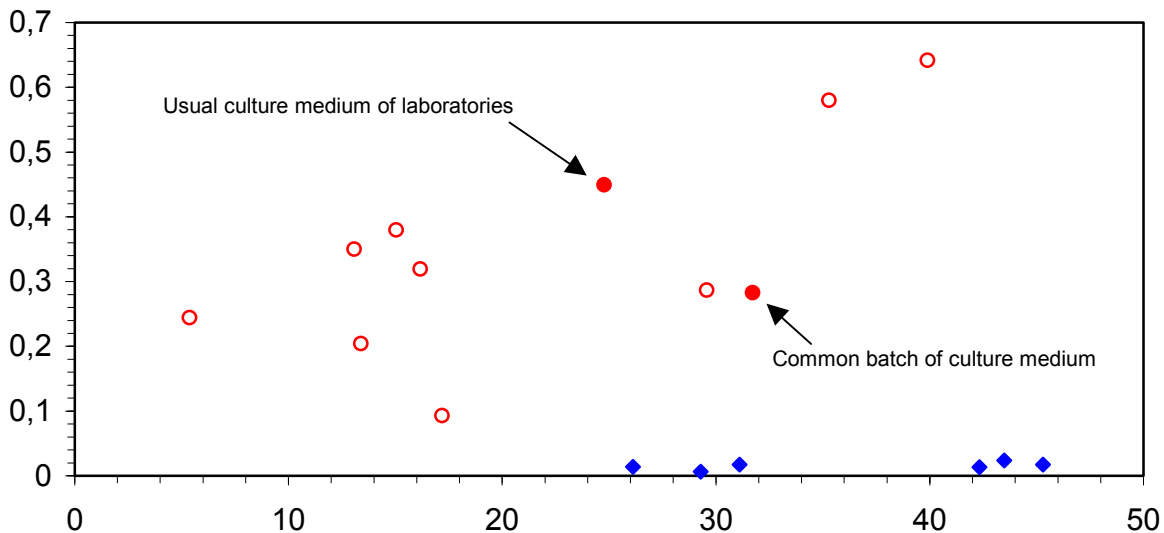
Interlaboratory trials organised by AGLAE between 1994 and 2005

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January 18, 2006

Enumeration of *Escherichia coli* in 100 ml

Test materials:

◆ Artificially contaminated waters ('spikes') ○ BioReference pastilles ● Trial 03M30.2 with BioReference pastilles



Each data point represents the result of an interlaboratory trial.

x-axis: mean number of *Escherichia coli* in 100 ml (arithmetic mean).

y-axis: squared coefficient of variation of the method ($CV_{\text{meth}}^2 = CV_R^2 - CV_{\text{Poisson}}^2$).

Notes:

1/ Several trials are not represented on the figure: the ones where the target organisms were not present in the test material and some trials with artificially contaminated waters for which the dispersion was limited to a Poisson distribution with a microbial load inferior to 10 in average per test portion volume.

2/ Trial 03M30.2 was a comparative trial aimed at quantifying the effect of the 'supplier of culture media' factor; the participants performed analyses on their culture medium and on a common medium (unique batch sent out for the trial).

Using artificially contaminated waters: $CV_{\text{meth}}^2 = 0,015$

Using BioReference pastilles: $CV_{\text{meth}}^2 = 0,344$

Expression of the uncertainty of measurement

Regulation value (in France) = absence of *Escherichia coli* in the 100 ml analysed;

therefore, expression of the uncertainty of measurement in terms of limit of detection (LD).

LD = 3,1 *Escherichia coli* in average in 100 ml if the germs are not stressed.

(note: LD with natural variation only is 3,0)

LD = 5,2 *Escherichia coli* in average in 100 ml if the germs are stressed.

(note: value obtained with BioReference pastilles)

Caution: the fact not to pick all the typical colonies for confirmation tests leads to higher LD.

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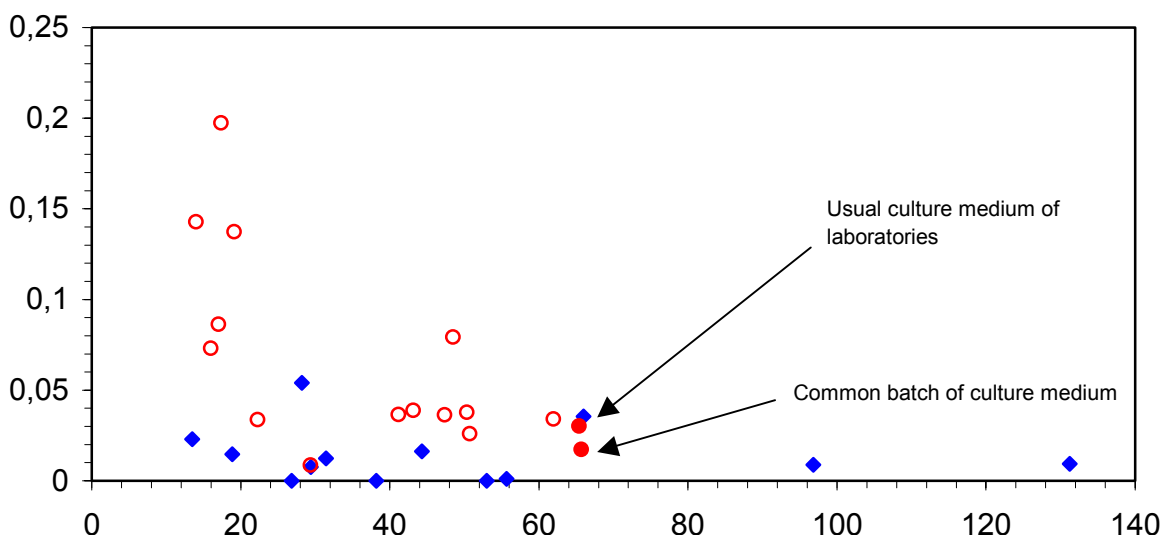
Interlaboratory trials organised by *AGLAE* between 1994 and 2005

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January 18, 2006

Enumeration of intestinal enterococci in 100 ml

Test materials:

◆ Artificially contaminated waters ('spikes') ○ BioReference pastilles ● Trial 03M30.2 with BioReference pastilles



Each data point represents the result of an interlaboratory trial.

x-axis: mean number of intestinal enterococci in 100 ml (arithmetic mean).

y-axis: squared coefficient of variation of the method ($CV_{\text{meth}}^2 = CV_R^2 - CV_{\text{Poisson}}^2$).

Notes:

1/ Several trials are not represented on the figure: the ones where the target organisms were not present in the test material and some trials with artificially contaminated waters for which the dispersion was limited to a Poisson distribution with a microbial load inferior to 10 in average per test portion volume.

2/ Trial 03M30.2 was a comparative trial aimed at quantifying the effect of the 'supplier of culture media' factor; the participants performed analyses on their culture medium and on a common medium (unique batch sent out for the trial).

Using artificially contaminated waters: $CV_{\text{meth}}^2 = 0,014$

Using BioReference pastilles: $CV_{\text{meth}}^2 = 0,069$

Expression of the uncertainty of measurement

Regulation value (in France) = absence of intestinal enterococci in the 100 ml analysed; therefore, expression of the uncertainty of measurement in terms of limit of detection (LD).

LD = 3,1 intestinal enterococci in average in 100 ml if the germs are not stressed.
(note: LD with natural variation only is 3,0)

LD = 3,3 intestinal enterococci in average in 100 ml if the germs are stressed.
(note: value obtained with BioReference pastilles)

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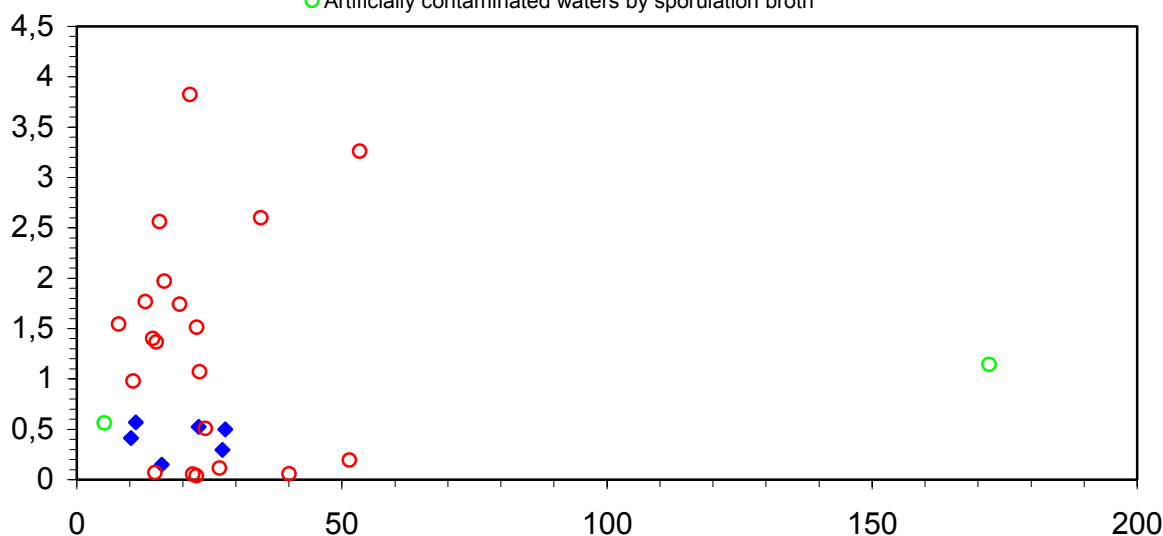
Interlaboratory trials organised by AGLAE between 1994 and 2005

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January 18, 2006

Enumeration of the spores of sulfite-reducing anaerobes in 50 ml

Test materials:

- ◆ Artificially contaminated waters by BioReference pastilles
- BioReference Pastilles
- Artificially contaminated waters by sporulation broth



Each data point represents the result of an interlaboratory trial.

x-axis: mean number of spores of sulfite-reducing anaerobes in 50 ml (arithmetic mean).

y-axis: squared coefficient of variation of the method ($CV_{\text{meth}}^2 = CV_R^2 - CV_{\text{Poisson}}^2$).

Notes:

1/ Several trials are not represented on the figure: the ones where the target organisms were not present in the test material.

Using artificially contaminated waters by BioReference pastilles:

$$CV_{\text{meth}}^2 = 0,409$$

Using BioReference pastilles:

$$CV_{\text{meth}}^2 = 1,331$$

Using artificially contaminated waters by sporulation broth:

$$CV_{\text{meth}}^2 = 0,853 \quad (2 \text{ trials})$$

Expression of the uncertainty of measurement

Regulation value (in France) = absence of spores of sulfite-reducing anaerobes in the 50ml analysed;

therefore, expression of the uncertainty of measurement in terms of limit of detection (LD).

LD = 5,9 spores of sulfite-reducing anaerobes in average in 50 ml if the germs are not stressed.
(note: LD with natural variation only is 3,0)

LD = 39,8 spores of sulfite-reducing anaerobes in average in 50 ml if the germs are stressed.
(note: value obtained with BioReference pastilles)

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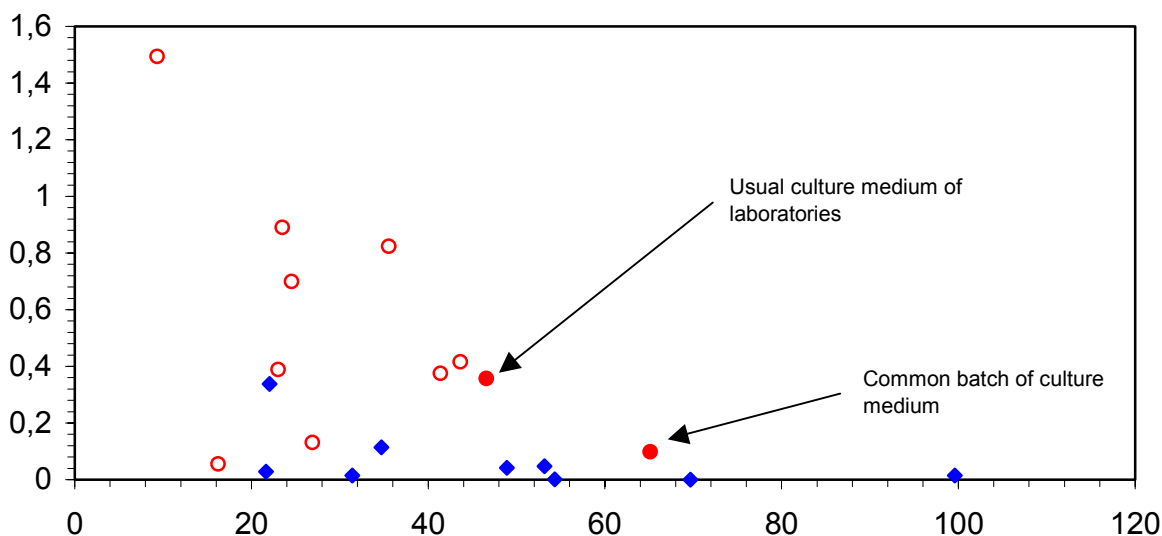
Interlaboratory trials organised by AGLAE between 1994 and 2005

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January 18, 2006

Enumeration of pathogenic staphylococci (coagulase +) in 100ml

Test materials:

◆ Artificially contaminated waters ('spikes') ○ BioReference pastilles ● Trial 03M31.2 with BioReference pastilles



Each data point represents the result of an interlaboratory trial.

x-axis: mean number of pathogenic staphylococci in 100 ml (arithmetic mean).

y-axis: squared coefficient of variation of the method ($CV_{\text{meth}}^2 = CV_R^2 - CV_{\text{Poisson}}^2$).

Notes:

1/ One trial is not represented due to anomalies detected in the quality of the test material sent to participants.

2/ Several trials are not represented on the figure: the ones where the target organisms were not present in the test material and some trials with artificially contaminated waters for which the dispersion was limited to a Poisson distribution with a microbial load inferior to 10 in average per test portion volume.

3/ Trial 03M31.2 was a comparative trial aimed at quantifying the effect of the 'supplier of culture media' factor; the participants performed analyses on their culture medium and on a common medium (unique batch sent out for the trial).

Using artificially contaminated waters:	$CV_{\text{meth}}^2 =$	0,067
Using BioReference pastilles:	$CV_{\text{meth}}^2 =$	0,586

Expression of the uncertainty of measurement

Regulation value (in France) = absence of coagulase + staphylococci in the 100 ml analysed; therefore, expression of the uncertainty of measurement in terms of limit of detection (LD).

LD = 3,3 coagulase + staphylococci in average in 100 ml if the germs are not stressed.
(note: LD with natural variation only is 3,0)

LD = 8,2 coagulase +staphylococci in average in 100 ml if the germs are stressed.
(note: value obtained with BioReference pastilles)

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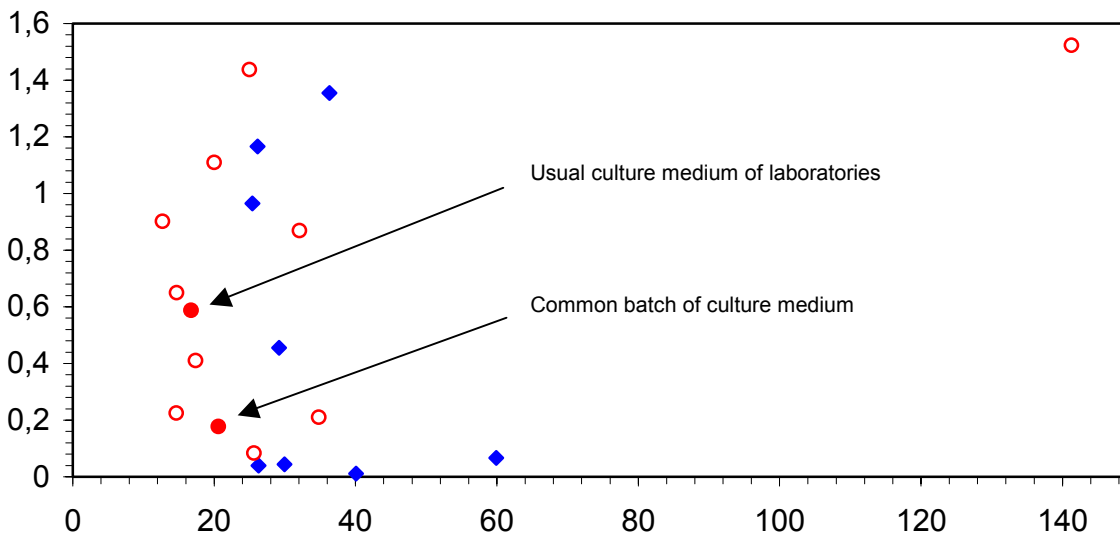
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January 18, 2006

Enumeration of *Pseudomonas aeruginosa* in 100 ml

Test materials:

◆ Artificially contaminated waters ('spikes') ○ BioReference pastilles ● Trial 03M31.2 with BioReference pastilles



Each data point represents the result of an interlaboratory trial.

x-axis: mean number of *Pseudomonas aeruginosa* in 100 ml (arithmetic mean).

y-axis: squared coefficient of variation of the method ($CV_{\text{meth}}^2 = CV_R^2 - CV_{\text{Poisson}}^2$).

Notes:

- 1/ One trial is not represented due to anomalies detected in the quality of the test material sent to participants;
- 2/ Several trials are not represented on the figure: the ones where the target organisms were not present in the test material and some trials with artificially contaminated waters for which the dispersion was limited to a Poisson distribution with a microbial load inferior to 10 in average per test portion volume.
- 3/ Trial 03M31.2 was a comparative trial aimed at quantifying the effect of the 'supplier of culture media' factor; the participants performed analyses on their culture medium and on a common medium (unique batch sent out for the trial).

Using artificially contaminated waters:	$CV_{\text{meth}}^2 =$	0,513
Using BioReference pastilles:	$CV_{\text{meth}}^2 =$	0,742

Expression of the uncertainty of measurement

Regulation value (in France) = absence of *Pseudomonas aeruginosa* in the 100 ml analysed; therefore, expression of the uncertainty of measurement in terms of limit of detection (LD).

LD = 7,1 *Pseudomonas aeruginosa* in average in 100 ml if the germs are not stressed.
(note: LD with natural variation only is 3,0)

LD = 11,1 *Pseudomonas aeruginosa* in average in 100 ml if the germs are stressed.
(note: value obtained with BioReference pastilles)