Pilot FRNA Bacteriophage Ring Trial

Introduction

The draft microbiological criteria (SANCO/4198/2001, rev. 5,3.2 2003) includes a requirement for assessment of FRNA bacteriophage levels in pre and post purification molluscs. In view of this, it is necessary that NRLs, and laboratories undertaking shellfish testing in member states, can demonstrate competence in the enumerative FRNA assay prior to the uptake of the legislation. The methodology for enumeration of FRNA bacteriophage (ISO 10705-1) is well standardised and robust, however, it is important for laboratories implementing any new procedure to undergo a period of training and consolidation, to achieve what is often referred to as "familiarity through use". As part of an ongoing programme to facilitate this aim, in autumn 2002 the CRL organised and hosted a series of FRNA training events. This training comprised both a practical and theoretical grounding in the assay. In addition, delegates were provided with a complete set of CRL standard operating procedures (SOP). Subsequently, a pilot FRNA ring trial was initiated, consisting of four distributions for analysis during January, February, March, and April 2003. A total of 15 participants took part in the pilot distributions. Eight of the participating laboratories were NRLs and 2 were laboratories within the member states nominated by the NRLs. Table 1 summarises the uptake of FRNA training and participation in the ring trial distributions.

Table 1. Ring Trial Summary FRNA

Country	**Delegates sent on FRNA training course**	**Ring trial material sent to these laboratories**	**Results returned to the CRL**			
			Vial 1	***Vial 2***	***Vial 3***	***Vial 4***
Austria	Yes	***XXX***	***XXX***	***XXX***	***XXX***	***XXX***
Belgium	Yes	NRL				
Denmark	Yes	NRL	√	√	√	√
Finland	Yes	NRL	√	√	√	√
France	Yes	NRL	√	√	√	√
Germany	Yes	***XXX***	***XXX***	***XXX***	***XXX***	***XXX***
Greece	No	Lab1	√	√	√	√
Ireland	Yes	Lab2	√	√	√	√
Italy	Yes	NRL	√	√	√	
Netherlands	No	NRL	√	√	√	√
Portugal	Yes	NRL	√	√	√	√
Spain	Yes	NRL	√	√	√	√
Sweden	Yes	NRL				
UK		NRL	√	√	√	√

XXX - countries not participating in the ring trial

Samples

Participants were requested to prepare the freeze dried ampoules according to the following instructions, and to analyse the rehydrated samples using their preferred methods.

Methodology for opening, preparation and dilution of FRNA bacteriophage ampoules

Opening of ampoules (wear protective gloves throughout the procedure.)

1. Care should be taken in opening the ampoule as the contents are in a vacuum.

2. Make a file mark on the ampoule near the middle of the cotton wool plug and either use a diamond pen to cut the glass or apply a red-hot glass rod to crack the glass.

3. Allow time for air, filtered by the plug, to seep into the ampoule and then gently remove the pointed top part. (If the pointed top part is snapped off suddenly the plug will be drawn to one end and may release fine particles of dried organisms into the air).

4. The plug may be impregnated with dried culture and should be regarded as dangerous to handle and removed with forceps.

Preparation of Samples

5. Flame the open end of the tube and add 1±0.1 ml of 0.1% peptone water to the ampoule.

6. Mix the contents carefully to avoid frothing or creating aerosols and leave the contents to rehydrate for a few minutes.

Dilution of Sample: serial dilute the sample as follows

7. Make the dilutions using 30-ml sterile universals; fill the first universal with 9.9 ml of 0.1% peptone water (1 part in 100). Fill seven further universals with 9.0 ml of 0.1% peptone water. *NB* All measurements must be exact.

8. Transfer 100 µl from the ampoule and pipette into 9.9 ml of 0.1% peptone water into the first universal, vortex (or shake) for 30±10 sec to mix the sample. Then using a 1.0 ml pipette transfer 1.0 ml of this sample and add to the next universal containing 9.0 ml of peptone water, mix well and repeat down the series until all dilutions have been carried out.

9. Keep the dilutions out of direct sunlight, to avoid any risk of damaging the viral particles in the suspensions.

10. The last three (i.e. 10^{-7}, 10^{-8}, 10^{-9}) of the eight universals contain the dilutions to be assayed in triplicate.

NB Parallel positive (MS2 NCO12487) and negative (0.1% peptone) controls must be used throughout in triplicate.

Quality control

The analyses were performed by the CRL on thirty samples prior to the examination periods. The results from these samples are referred to as the reference results, and were obtained using the CRL SOP (MFS SOP02 issue 5).

Analysis of results

The participants results were assessed by comparison with those returned by other participants and with the reference results. To avoid interference from extreme values (outliers) robust estimators are used to assess performance. Each reported result for the target dilution series (10^{-9}) was compared with the global centre (median),

calculated using all results from each distribution and including the reference results. A symmetrical robust estimator around the median (median absolute deviation (MAD)) (Staudte, R.G. & Sheather, S.J. 1990) was used to set upper and lower performance criteria. The MAD is defined as:
for data Yi i=1,...,n with M = median (Yi)

MAD = 1.4826 * median { abs(Yi - M)) }

where abs is the absolute value function.

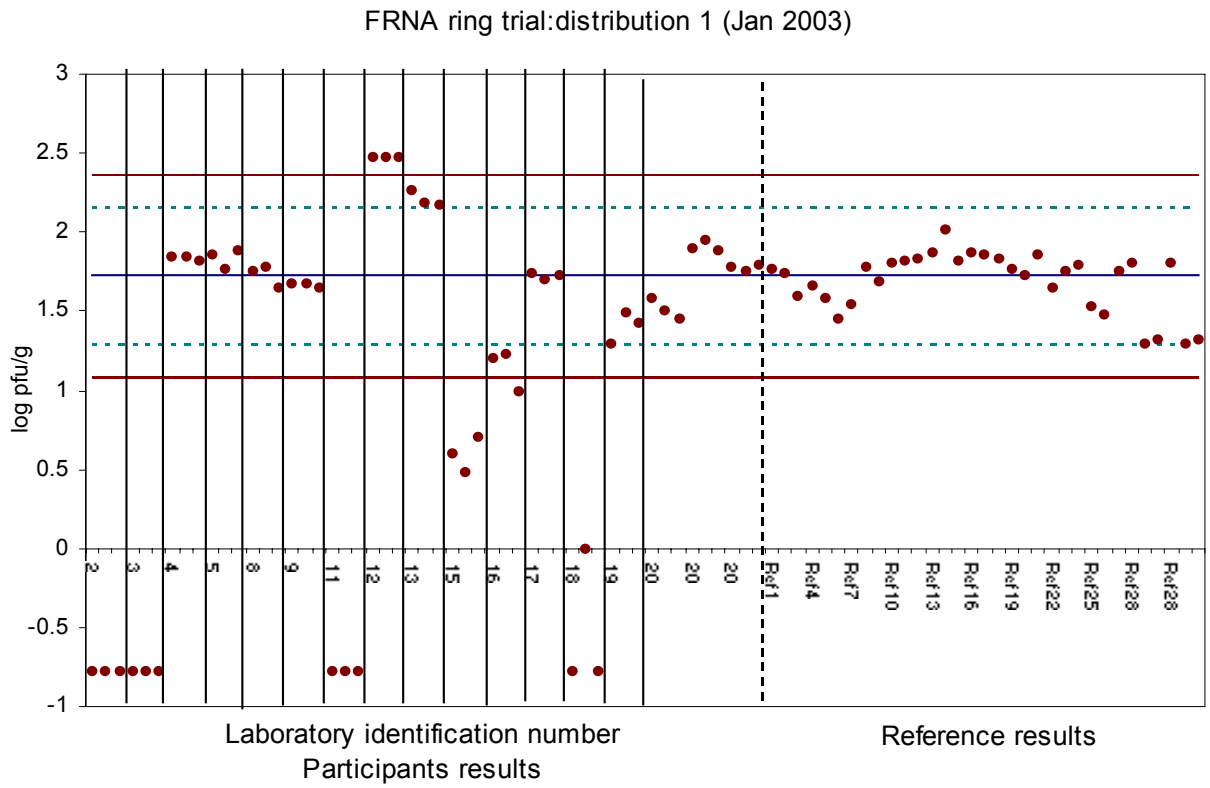
(The 1.4826 is used so that MAD matches standard deviation for normally distributed data.)

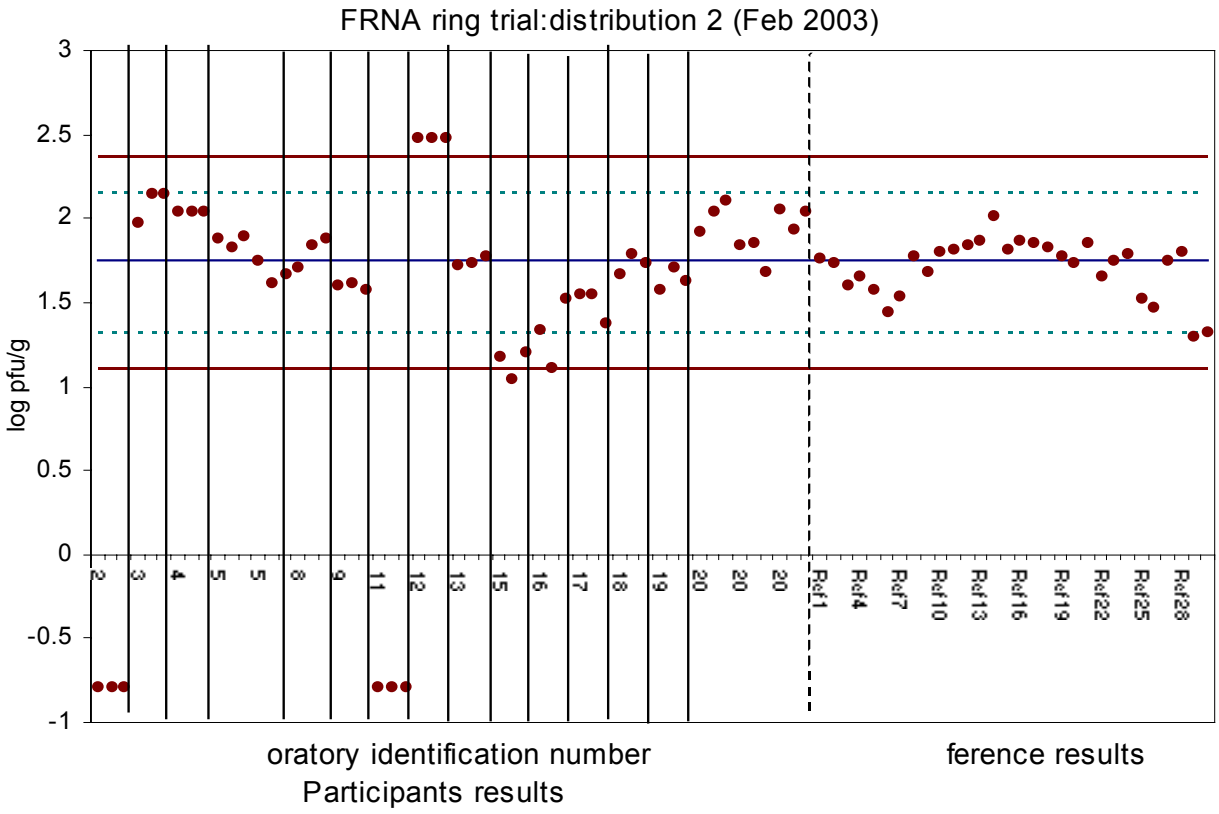
Results

Table 2: FRNA median and performance criteria (2/3 median absolute deviation -/+ median)

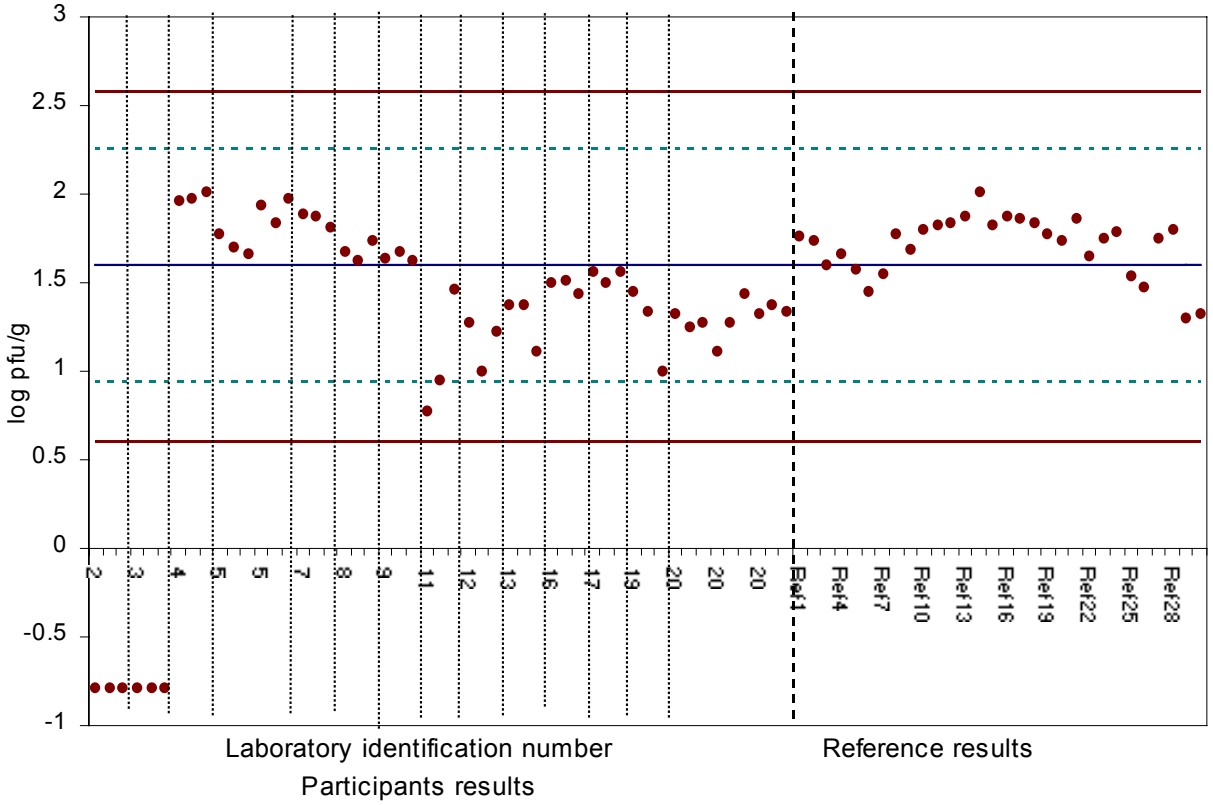
Log_{10} FRNA	Median	-2MAD	-3MAD	+2MAD	+3MAD
Reference results	**1.756**	**1.495**	**1.364**	**2.017**	**2.147**
Distribution 1 (vial 1) January 2003	**1.724**	**1.294**	**1.079**	**2.154**	**2.369**
Participants results	**1.771**	**1.479**	**1.332**	**2.063**	**2.209**
Distribution 2 (vial 2) February 2003	**1.748**	**1.389**	**1.210**	**2.107**	**2.287**
Participants results	**1.763**	**1.406**	**1.227**	**2.120**	**2.299**
Distribution 3 (vial 3) March 2003	**1.574**	**0.979**	**0.682**	**3.169**	**2.466**
Participants results	**1.690**	**1.219**	**0.984**	**2.161**	**2.396**
Distribution 4 (vial 4) April 2003	**1.771**	**1.497**	**1.360**	**2.045**	**2.182**
Participants results	**1.732**	**1.259**	**1.022**	**2.206**	**2.443**

The triplicate results reported by participants and the reference results for FRNA bacteriophage for the four distributions are plotted in Figures 1 through 4.

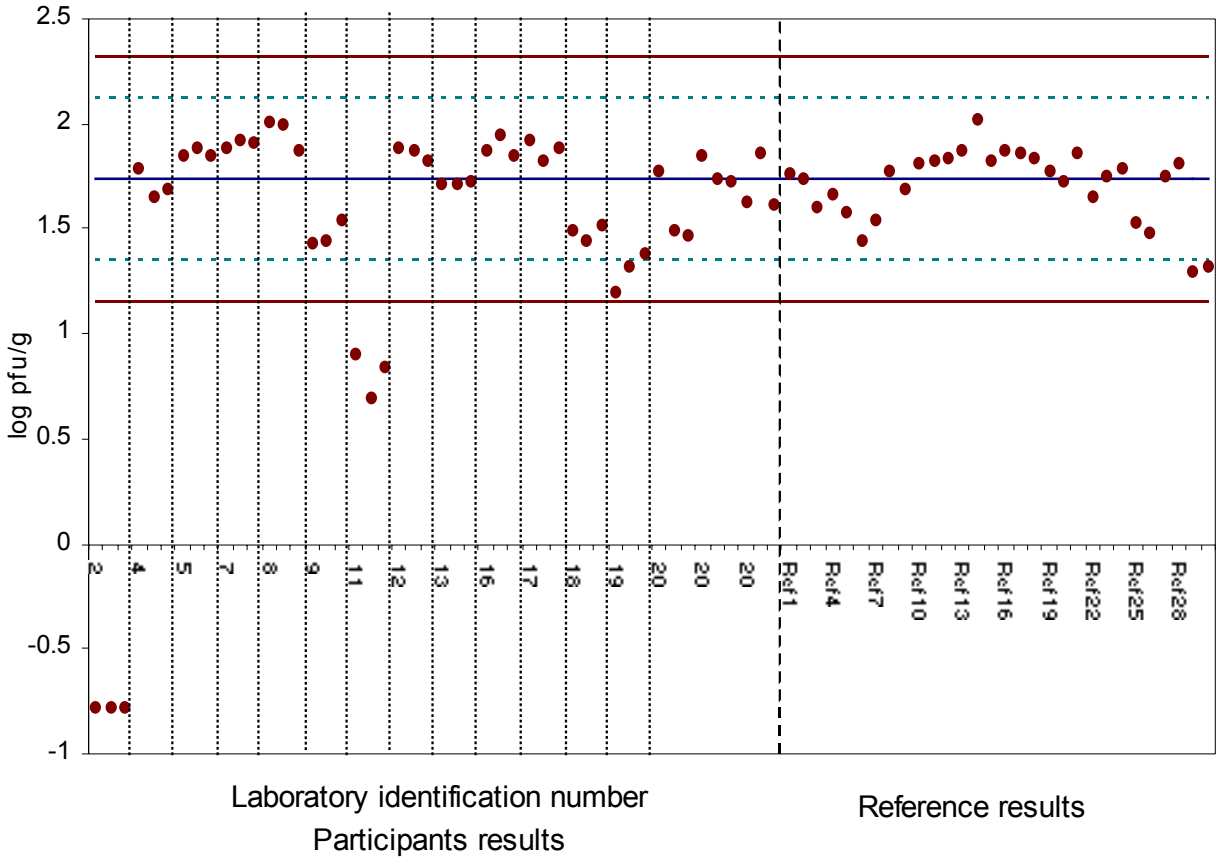

FRNA ring trial:distribution 1 (Jan 2003)
log pfu/g
3
2.5
2
1.5
1
0.5
0
-0.5
-1
2
3
4
5
8
9
11
12
13
15
16
17
18
19
20
20
20
Ref1
Ref4
Ref7
Ref10
Ref13
Ref16
Ref19
Ref22
Ref25
Ref28
Ref28
Laboratory identification number
Participants results
Reference results


FRNA ring trial:distribution 2 (Feb 2003)
log pfu/g
3
2.5
2
1.5
1
0.5
0
-0.5
-1
2
3
4
5
5
8
9
11
12
13
15
16
17
18
19
20
20
20
Ref1
Ref4
Ref7
Ref10
Ref13
Ref16
Ref19
Ref22
Ref25
Ref28
oratory identification number
Participants results
ference results

FRNA ring trial:distribution 3 (Mar 2003)



FRNA ring trial:disribution 4(Apr 2003)



Comments
(comments refer to NRLs only)

Distribution 1: Three laboratories reported triplicate negative results, one laboratory reported triplicate results greater than 3 median absolute deviations plus the median. One laboratory reported 2 result between 2 and 3 MAD + median. All other laboratories reported results within 2 MAD of the median.

Distribution 2: Two laboratories reported triplicate negative results, one laboratory reported triplicate results greater than 3 median absolute deviations plus the median. One laboratory reported 1 result between 2 and 3 MAD + median. All other laboratories reported results within 2 MAD of the median.

Distribution 3: Two laboratories reported triplicate negative results. One laboratory reported 2 result between 2 and 3 MAD + median. All other laboratories reported results within 2 MAD of the median.

Distribution 4: One laboratory reported triplicate negative results. One laboratory reported 3 results less than 3 median absolute deviations - the median. All other laboratories reported results within 2 MAD of the median.

One laboratory, that reported consistently negative results, experienced problems with transportation and vials arrived in poor conditions. The results of the distributions demonstrated a considerable improvement over the four month distribution series.