



European Community Reference laboratory
for monitoring bacteriological and viral
contamination of bivalve molluscs

Report on the Norovirus/Hepatitis A Ring Trial, 2008

CRL ring trial reference: RT 27 (NoV/HAV 2008)

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1. Introduction

In December 2008 the CRL organised a distribution of laboratory constructed samples in lenticule format for the detection of norovirus and hepatitis A virus.

2. Proficiency testing samples

2.1 Sample preparation and distribution

Laboratory constructed lenticules (RT 27 L1 to RT 27 L6) were prepared following the method of Codd *et al* (1998) with minor modifications. Hepatitis A virus (HAV), NoV genogroup I and II (Table 1) were prepared in phosphate buffered saline (PBS) and added at a 1:5 ratio to lenticulating fluid. The inoculated lenticulating fluid was aliquoted onto parafilm in 25µl volumes and placed in a desiccating chamber at 3±2°C for 1 week. After 1 week lenticules were transferred to <-15°C. Samples were packaged according to IATA regulations and distributed to twenty-eight participating laboratories by Worldnet shipping on the 1st December 2008. On receipt, participants were requested to store the samples at <-15°C prior to analysis during the week commencing the 8th December 2008.

Table 1: Source of viruses and designation

| Sample description | Source | Sequence type |
|------------------------|-----------------------------|---|
| Norovirus genogroup I | Faecal material | GI.4 capsid type; 96.2% sequence homology to Chiba virus (AB022679) |
| Norovirus genogroup II | Faecal material | GII.4 capsid type; 99.7% sequence homology to Isumi strain (AB295790) |
| Hepatitis A | Laboratory reference strain | strain HM175/43c |

2.2 Quality control at dispatch

Lenticules were tested prior to distribution to confirm virus presence and levels. Analyses were undertaken using CRL routine one-step RT-PCR method. The expected results and estimated quantities (geometric mean) for each lenticule are given in Table 2.

Table 2: Taqman™ expected results of RT 27 ring trial material

| Sample | Norovirus GI | GII | HAV |
|------------|----------------------------|----------------------------|----------------------------|
| RT 27 – L1 | - | + (3.3 x 10 ³) | - |
| RT 27 – L2 | - | - | - |
| RT 27 – L3 | + (3.6 x 10 ³) | - | + (1.3 x 10 ⁵) |
| RT 27 – L4 | + (2.6 x 10 ³) | + (1.7 x 10 ³) | + (1.2 x 10 ⁶) |
| RT 27 – L5 | - | - | + (7.8 x 10 ⁴) |
| RT 27 – L6 | + (4.9 x 10 ³) | - | - |

^a Geometric mean quantities expressed as genome copies per lenticule in parentheses

3.0 Results

3.1 Confidentiality of results

Each laboratory was provided with a personal identification number to preserve anonymity.

3.2 Reference results

Reference analyses were performed by the CRL on lenticules stored at <-15°C. Three randomly selected lenticules (L1-L6) were extracted in duplicate and RT-PCR (TaqMan™) was carried out

in triplicate. Box and whisker plots describing estimated target quantities derived from reference testing and expressed as genome copies per lenticule are given in Appendix I.

3.3 Analysis of results

Twenty-seven laboratories returned results. Participants' results (Table 3) were assessed as percentage relative sensitivity, specificity and accuracy for each determinant according to the following calculations:

Percentage relative sensitivity: Relative sensitivity (SE) = $\frac{TP}{(TP+FN)}$ x 100%

Percentage relative specificity: Relative specificity (SP) = $\frac{TN}{(TN+FP)}$ x 100%

Percentage relative accuracy: Relative accuracy (AC) = $\frac{TP+TN}{N}$ x 100%

Where TP = true positives
 FN = false negatives
 FP = false positives
 TN = true negatives
 N = total number of tests

Note: Participants' results were expressed as percentage concordance with intended results generated by the CRL. In this assessment presence/absence data was used and no consideration of quantitative measurements (Ct values) was made.

Table 3. Participants' results for all lenticules (L1 - L6)

| Lab ID number | GI | | | GII | | | HAV | | |
|---------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | SE | SP | AC | SE | SP | AC | SE | SP | AC |
| 2 | 100 | 100 | 100 | 100 | 75 | 83 | 100 | 100 | 100 |
| 3 | 100 | 100 | 100 | 100 | 50 | 67 | 100 | 67 | 83 |
| 7 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 9 | 33 | 67 | 50 | 100 | 50 | 67 | 33 | 67 | 50 |
| 10 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 11 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 15 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 17 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 19 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 21 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 24 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 25 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 27 | 33 | 33 | 33 | 100 | 100 | 100 | 100 | 100 | 100 |
| 32 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 33 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 35 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 37 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 39 | 67 | 100 | 83 | 50 | 100 | 83 | 100 | 100 | 100 |
| 41 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 44 | 33 | 100 | 67 | 0 | 75 | 50 | 0 | 100 | 50 |
| 47 | 67 | 100 | 83 | 100 | 100 | 100 | 100 | 100 | 100 |

| Lab ID number | GI | | | GII | | | HAV | | |
|---------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | SE | SP | AC | SE | SP | AC | SE | SP | AC |
| 48 | 100 | 100 | 100 | 100 | 100 | 100 | 67 | 100 | 83 |
| 83 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 94 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 114 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 133 | 100 | 100 | 100 | 100 | 100 | 100 | NE | NE | NE |
| 146 | 67 | 100 | 83 | 100 | 100 | 100 | 100 | 100 | 100 |

NE - Not examined

Lab 44 - Reported NoV presence or absence, and did not differentiate between genogroups.

Lab 133 - Did not test for HAV.

4. Conclusion and discussion

4.1 General comments

Twenty-seven laboratories (17 NRLs and 10 non-NRL and third country laboratories) returned results. Laboratory 133 did not examine for HAV. Laboratory 44 used a non-discriminatory method for detection of NoV. Laboratory 113 did not return results. Results reported to the CRL are shown in Appendices I, II and III.

4.2 Discussion

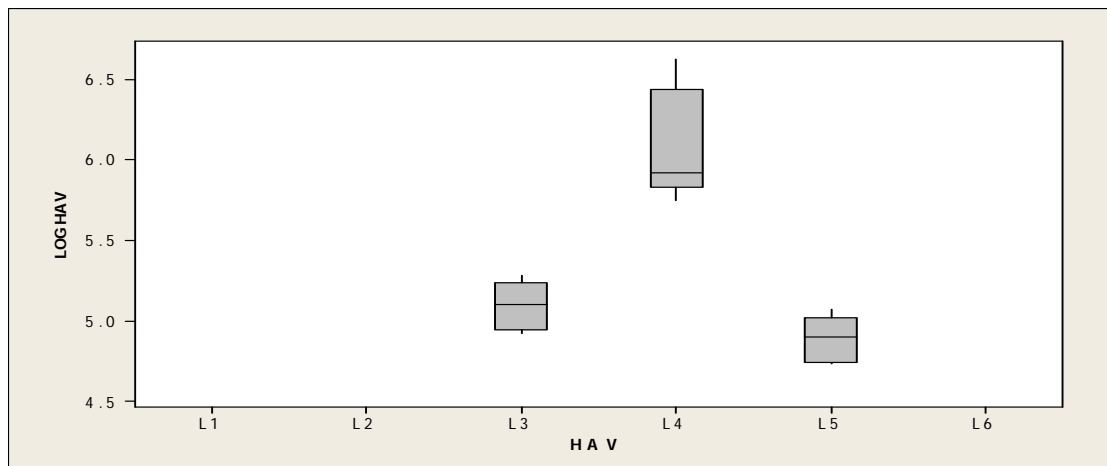
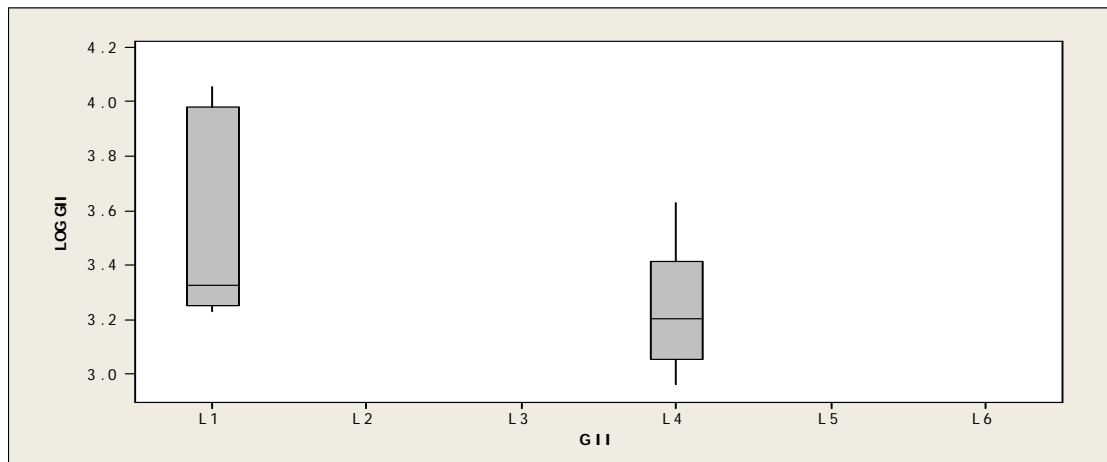
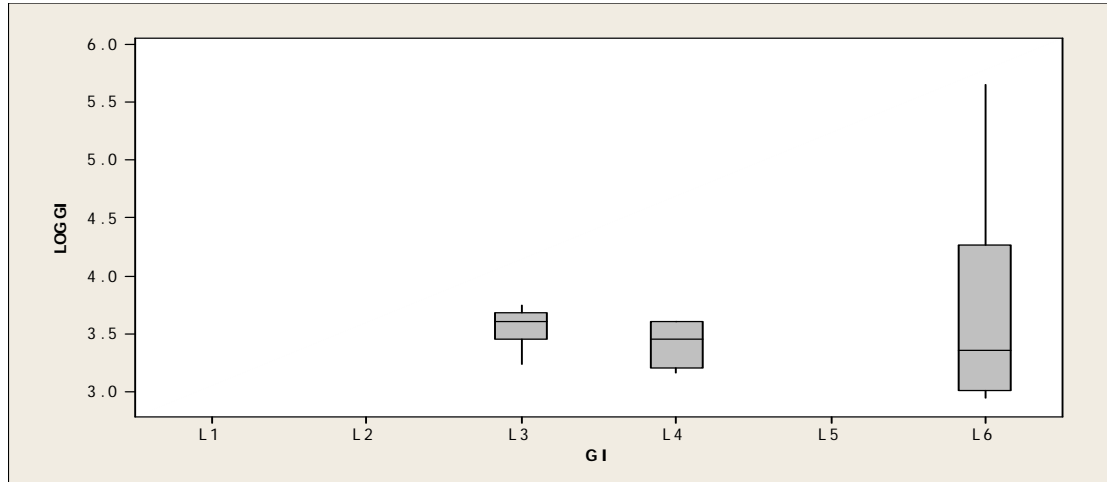
Approximately a third (67%) of participating laboratories obtained intended results for all lenticules, as determined by CRL reference designations. For individual viruses, 41%, 48% and 72% of laboratories returned intended results for norovirus GI, GII and hepatitis A respectively. One laboratory did not examine for HAV. The false positive reporting rates for GI, GII and HAV were 4%, 6% and 6% respectively. The false negative reporting rates for GI, GII and HAV were 11%, 6% and 11% respectively. Eighteen laboratories (67%) returned semi-quantitative data expressed at C_t values (Appendices II, III and IV). Seven laboratories returned quantitative data expressed as detectable genome copies per lenticule (Appendix V).

5 References

Codd AA, Richardson IR, Andrews N. 1998. Lenticules for the control of quantitative methods in food microbiology. *J Appl Microbiol.* **85(5)**:913–7.

Appendix I - Reference results

CRL Reference results displayed as box and whisker plots of detectable genome copies per 25µl lenticule.



Appendix II - Participants' presence/absence results for each lenticule

| Lab ID | Lenticule 1 | | | Lenticule 2 | | | Lenticule 3 | | | Lenticule 4 | | | Lenticule 5 | | | Lenticule 6 | | |
|--------|-------------|-----|-----|-------------|-----|-----|-------------|-----|-----|-------------|-----|-----|-------------|-----|-----|-------------|-----|-----|
| | GI | GII | HAV | GI | GII | HAV | GI | GII | HAV | GI | GII | HAV | GI | GII | HAV | GI | GII | HAV |
| | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 2 | - | + | - | - | - | - | + | - | + | + | + | + | - | + | + | + | - | - |
| 3 | - | + | - | - | - | + | + | + | + | + | + | + | - | - | + | + | + | + |
| 7 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 9 | - | + | - | - | + | + | - | - | - | - | + | - | + | + | + | + | + | - |
| 10 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 11 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 15 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 17 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 19 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 21 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 24 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 25 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 27 | - | + | - | + | - | - | + | - | + | - | + | + | + | - | + | + | - | - |
| 32 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 33 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 35 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 37 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 39 | - | - | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 41 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 44 | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - |
| 47 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 48 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | - | + | - | - |
| 83 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 94 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 114 | - | + | - | - | - | - | + | - | + | + | + | + | - | - | + | + | - | - |
| 133 | - | + | nt | - | - | nt | + | - | nt | + | + | nt | - | - | nt | + | - | nt |
| 146 | - | + | - | - | - | - | - | - | + | + | + | + | - | - | + | + | - | - |

Yellow denotes false positives, Red denotes false negatives.

Appendix III - Participants' reported GI and GII C_t values for each lenticule

| Lab. ID. | Lenticule 1 | | Lenticule 2 | | Lenticule 3 | | Lenticule 4 | | Lenticule 5 | | Lenticule 6 | |
|----------|-------------|--------------|-------------|--------|--------------|--------|--------------|--------------|-------------|--------|--------------|--------------|
| | NV GI | NV GII | NV GI | NV GII | NV GI | NV GII | NV GI | NV GII | NV GI | NV GII | NV GI | NV GII |
| 2 | - | + | - | - | + | - | + | + | - | + | + | - |
| 3 | - | 34.26, 33.29 | - | - | 39.37, 39.37 | 37.55 | 40.67, 40.78 | 35.43, 34.91 | - | - | 40.7, 40.37 | 38.73, no Ct |
| 7 | - | + | - | - | + | - | + | + | - | - | + | - |
| 9 | - | + | - | + | - | - | - | + | + | + | + | - |
| 10 | - | 37.59, 37.6 | - | - | 36.43, 36.29 | - | 36.82, 36.49 | 36.49, 35.03 | - | - | 37.09, 37.96 | - |
| 11 | - | 38.15 | - | - | 39.18 | - | 39.07 | 40.48 | - | - | 38.01 | - |
| 15 | - | 33 | - | - | 40 | - | 39 | 34 | - | - | 38 | - |
| 17 | - | 31.24 | - | - | 37.62 | - | 36.96 | 30.19 | - | - | 37.86 | - |
| 19 | - | 35.6 | - | - | 37 | - | 37.4 | 36.2 | - | - | 39.8 | - |
| 21 | - | 36.8 | - | - | 38.7 | - | 37.7 | 37.1 | - | - | 40 | - |
| 24 | - | 32.78 | - | - | 37.17 | - | 37.31 | 33.86 | - | - | 36.57 | - |
| 25 | - | 33.79, 33.84 | - | - | 39.69, 39.94 | - | 40.39, 40.11 | 37.49, 37.59 | - | - | 40.63, 40.28 | - |
| 27 | - | 45.8, 43.5 | 42.2, 41.9 | - | 45.4, 44.5 | - | - | 47.9, 43.4 | 50, 50.3 | - | - | - |
| 32 | - | 36.9 | - | - | 39.6 | - | 39.4 | 38 | - | - | 40.2 | - |
| 33 | - | + | - | - | + | - | + | + | - | - | + | - |
| 35 | - | + | - | - | + | - | + | + | - | - | + | - |
| 37 | - | + | - | - | + | - | + | + | - | - | + | - |
| 39 | - | - | - | - | + | - | + | 33, 35 | - | - | - | - |
| 41 | - | 31.32 | - | - | 36.62 | - | 37.37 | 31.78 | - | - | 36.21 | - |
| 44 | - | - | - | - | + | + | - | - | - | - | - | - |
| 47 | - | + | - | - | + | - | + | + | - | - | - | - |
| 48 | - | 35.1 | - | - | 39.9 | - | 40.5 | 34.9 | - | - | 41.9 | - |
| 83 | - | 34.8 | - | - | 35.8 | - | 36.5 | 35.3 | - | - | 35.3 | - |
| 94 | - | 29.47 | - | - | 34.66 | - | 33.77 | 28.99 | - | - | 33.4 | - |
| 114 | - | 30.34 | - | - | 33.09 | - | 33.39 | 31.4 | - | - | 33.44 | - |
| 133 | - | 36.82 | - | - | 36.55 | - | 34.34 | 36.7 | - | - | 36.9 | - |
| 146 | - | 36.63, 36.27 | - | - | - | - | 38.82, 39.71 | 36.35, 37.38 | - | - | 40.73, 40.72 | - |

Yellow denotes false positives, Red denotes false negatives.

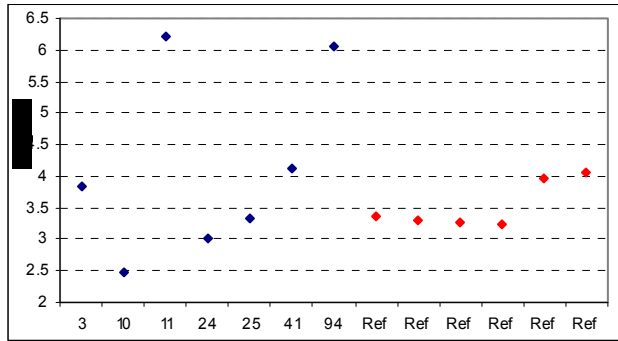
Appendix IV - Participants' reported HAV C_t values for each lenticule

| Lab. ID. | Lenticule 1 HAV | Lenticule 2 HAV | Lenticule 3 HAV | Lenticule 4 HAV | Lenticule 5 HAV | Lenticule 6 HAV |
|----------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| 2 | - | - | + | + | + | - |
| 3 | - | 39.62, no Ct | 33.03, 32.31 | 32.3, 33.05 | 34.16, 33.73 | - |
| 7 | - | - | + | + | + | - |
| 9 | - | + | - | - | + | - |
| 10 | - | - | 31.67, 31.82 | 31.54, 31.79 | 32.58, 33.02 | - |
| 11 | - | - | 36.14 | 36.29 | 37.09 | - |
| 15 | - | - | 36 | 34.5 | 35.5 | - |
| 17 | - | - | 31.01 | 31.85 | 32.77 | - |
| 19 | - | - | 32.3 | 31.8 | 33.4 | - |
| 21 | - | - | 35.7 | 35.4 | 37.8 | - |
| 24 | - | - | + | + | + | - |
| 25 | - | - | 31.42, 31.95 | 32.72, 32.48 | 33.39, 33.28 | - |
| 27 | - | - | 44.5, 43.4 | 46.3, 43.4 | 45.1, 45.6 | - |
| 32 | - | - | 31.1 | 30.9 | 32.4 | - |
| 33 | - | - | + | + | + | - |
| 35 | - | - | + | + | + | - |
| 37 | - | - | + | + | + | - |
| 39 | - | - | 27, 29 | 28, 30 | 31, 32 | - |
| 41 | - | - | 29.27 | 29.87 | 30.14 | - |
| 44 | - | - | - | - | - | - |
| 47 | - | - | 33.9 | 33.6 | 35.4 | - |
| 48 | - | - | 40.3 | 39.3 | - | - |
| 83 | - | - | 31.9 | 32.1 | 33.3 | - |
| 94 | - | - | 25.26 | 25.96 | 26.95 | - |
| 114 | - | - | 27.03 | 26.36 | 27.85 | - |
| 133 | nt | nt | nt | nt | nt | nt |
| 146 | - | - | 34.24, 34.54 | 33.48, 33.18 | 35.88, 36.08 | - |

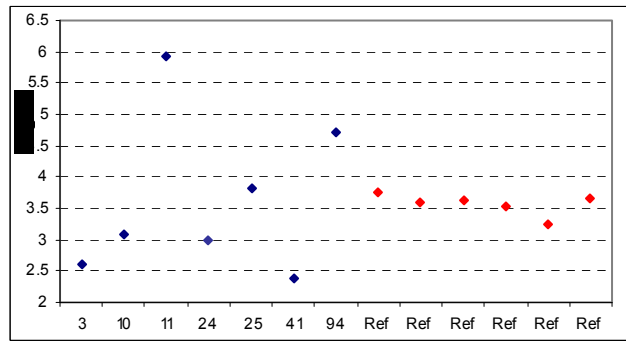
Yellow denotes false positives, Red denotes false negatives.

Appendix V – Participants' and reference quantities per lenticule for each target.

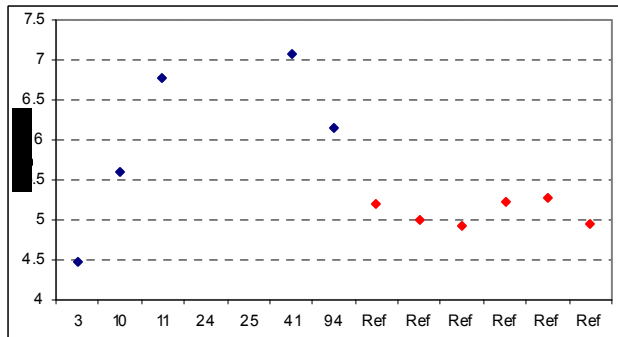
Lenticule 1 - GII



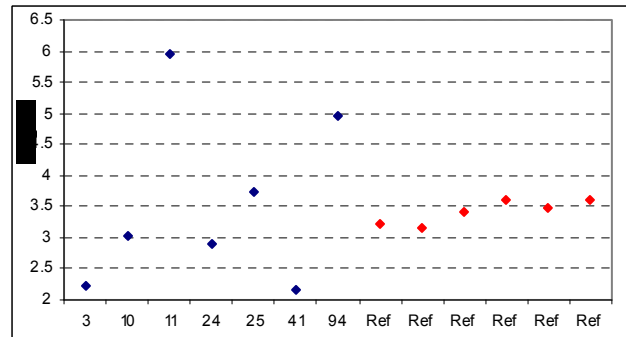
Lenticule 3 - GI



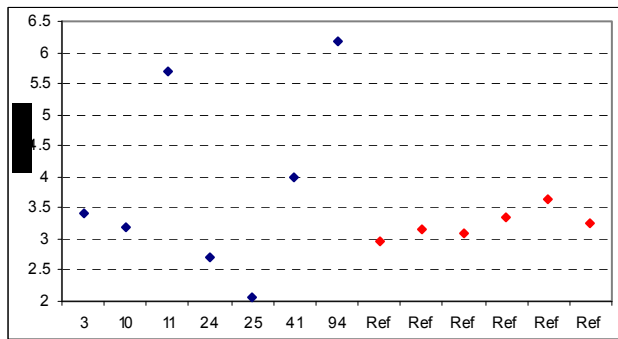
Lenticule 3 - HAV



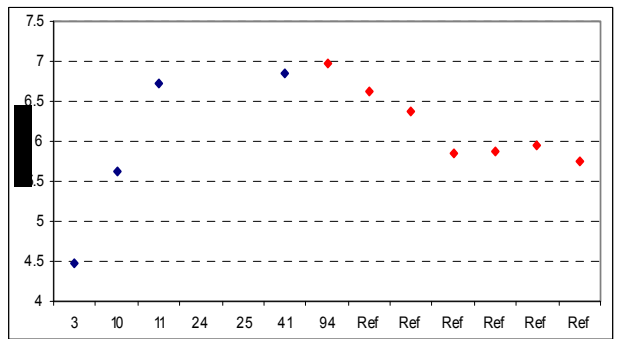
Lenticule 4 - GI



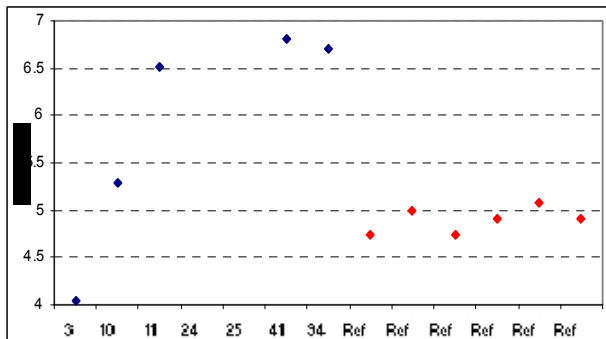
Lenticule 4 - GII



Lenticule 4 - HAV



Lenticule 5 - HAV



Lenticule 6 - GI

