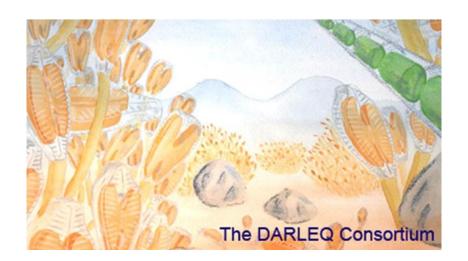
# DARLEQ: Diatom Assessment of River and Lake Ecological Quality Version 2.0

# **User Guide**



# Software for Freshwater Status Classification using benthic diatoms

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## 1. Introduction

DARLEQ 2 is a Microsoft Windows<sup>®</sup> program for the assessment of river and lake ecological status using diatoms. The program takes input data in Microsoft Excel<sup>®</sup> format, performs some simple checks to validate data fields, links the list of diatom codes in the sample data to the DARLEQ master taxon list, and calculates various metrics, EQR and status class for each sample. These steps are described in detail below.

DARLEQ 2 can calculate Trophic Diatom Index TDI4 and TDI3 scores for river samples, Lake Trophic Diatom Index LTDI2 and LTDI1 scores for lake samples, and Diatom Acidification Metric (DAM) scores for all samples. Details of the TDI / LTDI metrics, algorithm and derivation of the status class boundaries for rivers are given in Kelly et al. (2008) and for lakes in Bennion et al. (2014). Details of the DAM acidification metric is described in Juggins & Kelly (2012). Additional references to these methods may be found at the end of this document.

## 2. Data format

Example datasets in Excel format for TDI/LTDI/DAM river/lake samepls are included with DARLEQ2 and installed in the folder C:\Program Files (x86)\DARLEQ Consortium\DARLEQ 2 Diatom Tool\TestData. The date may be opened directly in Excel by clicking the "Open Example Dataset button":

Input data must be in a Microsoft Excel workbook, in either xls or xlsx format. Required data and layout are slightly different for river and lake samples. Figure 1 shows the required format for performing TDI calculations for river samples.

	Α	В	С	D	Е	F	G
1	SampleID		SPR001	AUT001	SPR002	AUT002	SPR003
2	SiteID		36082	36082	34649	34649	36073
3	SampleDate		14/04/2004	21/09/2004	02/04/2004	21/09/2004	02/04/2004
4	Alkalinity		242	242	408	408	213
5	Stream		KENNET	KENNET	LAMBOURN	LAMBOURN	LAMBOURN
6	Reach		Hambridge F	Hambridge Rd., No	A4 Newbury	A4 Newbury	At Gauging Station
7	AC023A	Achnanthes conspicua var. conspicua	0	0	0.332226	0	0
8		Achnanthes exigua	0	0	0	0	0
9	AC149A	Achnanthes kranzii	0	0	0	0	0
10	AC083A	Achnanthes laevis	0	0	0	0	0
11	AC095A	Achnanthes minuscula	0	0	0	0	0
12	AC143A	Achnanthes oblongella	0	0	0	0	0
13	AC120A	Achnanthes semiaperta	0	0	0	0	0
14		Achnanthes sp.	0	0	0	0	0
		Achnanthes ventralis	0	0	0	0	0
16	ZZZ912	Achnanthidium biasolettiana	0	0	0	0	2.15385
		Achnanthidium microcephalum	0.31746	0	0	0	0
18	ZZZ835	Achnanthidium minutissimum type	17.1429	1.28617	7.30897	2.11082	27.3846
19	ZZZ911	Achnanthidium subatomus	0	0	0	0	0
20	AP001A	Amphipleura pellucida	0	0	0	0	0.307692
21	AM039A	Amphora delicatissima	0	0	0	0	0
22	AM010A	Amphora fogediana	0	0	0	0	0
23	AM013A	Amphora inariensis	1.26984	0	1.3289	0	0

Figure 1: Example format for river diatom samples

The first four header rows are mandatory and must contain the following information:

- Row 1: Sample identifier short numerical or alphanumeric code to uniquely identify the sample. This field cannot be empty an empty cell indicates the end of data.
- Row 2: Site identifier short numerical or alphanumeric code to uniquely identify the site. This code will be used to aggregate multiple samples when calculating confidence of class for a site.
- Row 3: Sample Date in Day/Month/Year format. Missing dates are set to "Spring" for the purposes of classification using TDI3 and samples flagged with a warning.
- Row 4: Mean annual alkalinity (or best available estimate) in mg l<sup>-1</sup> (CaCO<sub>3</sub>). Missing values are set to 100 mg l<sup>-1</sup> for the purposes of classification and samples flagged with a warning. Alkalinity values outside the range of the site prediction algorithm are set to the appropriate limit (6 or 150 mg l<sup>-1</sup> for TDI3 and 5 or 250 mg l<sup>-1</sup> for TDI4).
- Row 5 ...n: Further option sample descriptors such as river name, reach name etc.

  These data are not used by the program but will be reproduced in the output.

Identifiers for each row of the sample header information should be listed in column 1. Diatom data then follow the header information and may be in count or percentage format. The first column must contain the taxon code in either NBS or DiatCode (<a href="http://www.ecrc.ucl.ac.uk/content/view/312/127/">http://www.ecrc.ucl.ac.uk/content/view/312/127/</a>) format. The codes in this column are used to link the data to the DARLEQ master taxon list and ecological information and cannot be empty – an empty cell indicate the end of the data. The second column can be blank or contain an optional taxon name. This is not used by the program but is useful in identifying errors in the data. Empty (blank) cells in the count or percentage data matrix will be read as zero. A full list of diatom codes (either NBS or DiatCodes) can be viewed by using the "View Master Taxon List" button:

If the Diatom Acidification Metric (DAM) is to be calculated, rows 5 and 6 must contain estimates of mean annual Calcium and DOC concentrations, in  $\mu$ eq I<sup>-1</sup> and mg I<sup>-1</sup> respectively. Figure 2 shows an example formatted for calculation of TDI and DAM. Note that if only DAM scores are required the Alkalinity field may be left blank. Sample Date is not used for calculating DAM and may be left blank.

	Α	В	С	D	Е	F	G	Н	1	J
1	Sampleld		UK002_90	UK002_91	UK002_92	UK003_90	UK003_91	UK003_92	UK009_90	UK009_91
2	Site		UK002	UK002	UK002	UK003	UK003	UK003	UK009	UK009
3	Date		1990	1991	1992	1990	1991	1992	1990	1991
4	Alkalinity									
5	Calcium		43.66	40.67	44.06	55.89	58.38	57.88	47.55	53.39
6	DOC		2.75	1.66	2.4	3.4	3.6	3.4	1.7	1.73
7	AC083A	Achnanthes laevis	0.0	0.0	0.6	0.0	1.3	0.0	0.0	0.0
8	AC143A	Achnanthes oblongella	0.6	1.0	1.2	49.5	56.4	84.7	0.0	0.3
9	AC148A	Achnanthes modestiformis	0.0	1.0	1.2	0.0	0.0	0.0	0.0	0.0
10	AC9999	Achnanthes sp.	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.0
11	BR001A	Brachysira vitrea	0.0	0.0	0.0	3.7	0.0	0.3	0.0	0.3
12	BR006A	Brachysira brebissonii fo brebissonii	0.0	0.0	0.0	0.0	0.0	0.0	12 8	14.9

Figure 2: Example format for river diatom samples for TDI and DAM calculations

	Α	В	С	D	E	F	G	Н	
1	Sampleld		ACHNAU4R	ACHNSP4P	ACHNSP4R	ACHNSU4R	AILSAU4R	AILSSP4R	AILSSU4R
2	SiteId		14403	14403	14403	14403	11338	11338	11338
3	SampleDa	te	08/11/2004	15/04/2004	15/04/2004	07/09/2004	04/11/2004	05/05/2004	30/06/2004
4	Туре		MA						
5									
6	AC001A	Achnanthes lanceolata	0.26178	0	0.455063	0	0	0	0
7	ACOO6A	Achnanthes clevei	0.26178	0.552486	0	0	0	0	0
8	AC007A	Achnanthes oestrupii	0.52356	0	0	0	0	0	1.58228
9	AC013A	Achnanthes minutissima	42.1466	26.2431	45.2787	56.0606	10.4938	45.0581	56.6456
10	AC016A	Achnanthes delicatula	0	0	0	0	0	0	0
11	AC022A	Achnanthes marginulata	0	0	0	0	0.308642	3.19767	0.949367
12	AC023A	Achnanthes conspicua	0	0	0	0	0	0	0
13	AC025A	Achnanthes flexella	0	0	0	0	0.308642	0	0.632911
14	AC034A	Achnanthes suchlandtii	0	0	0	0	0	0	1.26582
15	AC035A	Achnanthes pusilla	0	0.828729	0.227531	0	0	2.90698	1.58228
16	AC044A	Achnanthes levanderi	0	0	0	0	0	0.872093	0
17	AC049A	Achnanthes ploenensis	0	0	0	0	0	0	0
18	AC083A	Achnanthes laevis	0	0	0	0	0	0	0
19	AC134A	Achnanthes helvetica var. alpina	0	0	0.227531	0	0	4.06977	0.949367
20	AC143A	Achnanthes oblongella	0	0	0	0	0	0	7.91139
21	AC161A	Achnanthes ventralis	0	0	0	0	0	0.290698	0
22	AC170A	Achnanthes joursacense	0	0	0	0	0	0	0

Figure 3: Example format for lake diatom samples

The required format for lake samples is shown in Figure 3. This is exactly the same as for river data except that the fourth row must contain a code indicating lake type according to the GB lake typology alkalinity classes (Table 1). Marl lakes are included in the high alkalinity (HA) group. Peat and brackish lakes are not covered by the tool. Sample date for lake samples is not used in the class calculations and can contain missing values.

Table 1: Lake types used in the tool

Code	Alkalinity					
	µeq l⁻¹	mg CaCO₃ l <sup>-1</sup>				
LA (Low Alkalinity)	< 200	< 10				
MA (moderate Alkalinity)	200 – 1000	10 – 50				
HA (High Alkalinity)	> 1000	> 50				

# 3. Running the program

Data are input by clicking on the File Open button on the toolbar or selecting File -> Open from the menu. After selecting the appropriate Excel file the program will read the file and launch the Select worksheet dialog box.

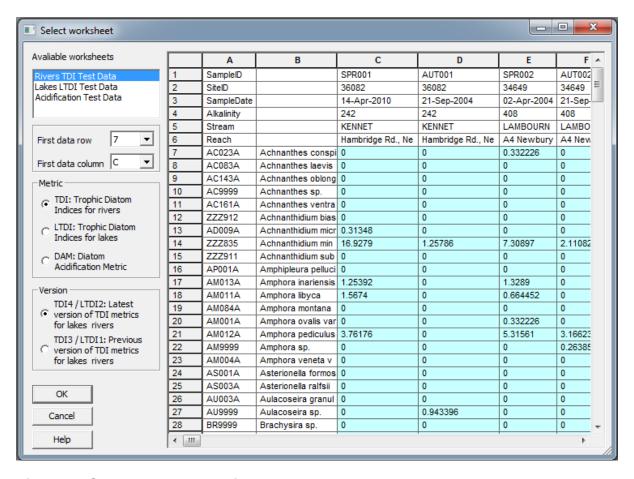


Figure 4: Select worksheet dialog box

Select the correct worksheet from the list on the left of the dialog box. DARLEQ2 tries to "guess" how many rows of optional sample information are present (if any) and highlights the area contain the *Diatom Count Data* in blue. If this is wrong simply click on the top left corner of the diatom count data. Once the correct area is highlighted select the Metric (TDI, LTDI or DAM) click OK. The program defaults to the latest version of the metrics (TDI4 and LTDI2). If you want to calculate an earlier version change the version to TDI3/LTDI1.

DARLEQ2 will then check the data for missing or bad values and issue a warning if sample dates or alkalinity values are missing (for river data), or if calcium or DOC values are missing for DAM calculations. If lake types are missing (for lake samples) the program will issue and error and ask you to correct the data and try again.

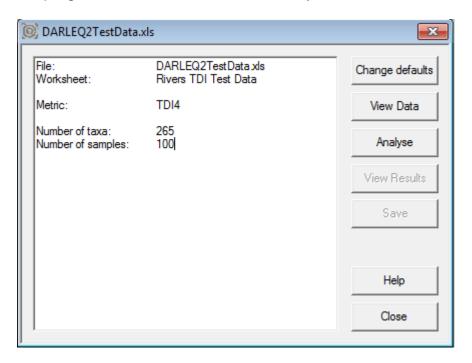


Figure 5: Analysis summary

When data have been read and checked by the program the Analysis Summary dialog box will appear. Click Analyse to calculate the status classification.

# 4. Understanding the output

DARLEQ2 will automatically determine the type of taxon coding (NBS or DiatCode) and attempt to link the taxon codes in the input data with those in its master taxon list. If any taxa present in the input data are not found in the master taxon list a warning is given in the Analysis Summary dialog box. The unmatched taxa can be identified by viewing the data (see below). A list of unmatched taxa is also written to the output file.

Results of the analysis can be viewed by clicking the View Results button. The format of the result is illustrated in Figures 6 and 7. For TDI and LTDI the results will usually contain two sheets identified by a tab on the bottom right and labelled Sample Summary and Uncertainty.

**Sample Summary** – this sheet contains results for each sample. First, the sample information given in the original input file is repeated, and then results of the analysis are listed as follows for each metric:

Sample sum: Sum of the counts or percentages of all taxa in a sample.

Sum TDI4: Sum of the counts or percentages for all taxa in a sample that are

matched to taxa in the master taxon list and included in the TDI calculation. If all taxa are matched this will be the same as the Sample sum. Comparison of these two fields will indicate if there are important taxa present in the sample but not included in the

status calculations.

TDI4: TDI4 score for each sample using the TDI4 taxon scores for

rivers.

eTDI4: Expected TDI4 score for each sample according to typology

(lakes) or site-specific prediction (rivers).

EQR: EQR for each sample based on predicted TDI4 for observed

alkalinity and season (rivers) or lake type.

Class: Status class based on EQR.

This examples uses TDI4. The Sample Summary for other metrics is identical.

After the metric and classification fields a series of summary fields are listed containing the percentage of various groups of diatoms:

% Planktic: Percentage of planktic diatoms in the sample. These are

excluded from the status calculations.

% Motile: Percentage of the motile diatoms in the sample.

%OrganicTolerant: Percentage of organic pollution tolerant diatoms in the sample.

%Saline: Percentage of diatoms tolerant of slightly saline waters.

Comments: List of any warning messages generated during calculations for

individual samples.

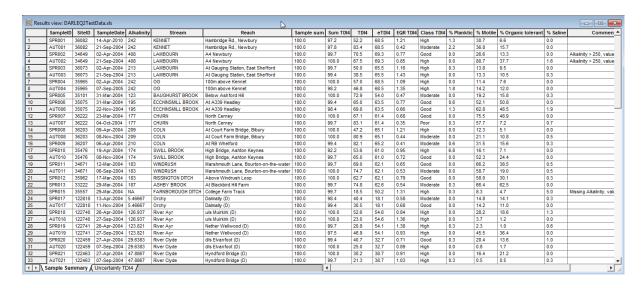


Figure 6: Sample Summary output for TDI4

**Uncertainty** – multiple samples from each site are combined and an uncertainty analysis is performed using the mean EQR and number of samples according to Kelly et al. (2009):

Site code: Unique site code taken from row 2 of the input data.

No. Samples Number of samples for site used in calculation of mean and CoC.

No. missing 
Number of samples for a site that have missing EQRs. This

should be zero.

Mean EQR Mean EQR for each site.

Class Status class based on mean EQR.

CoC High Confidence that the site belongs to status class "high", etc.

RoM Rick of misclassification for predicted class.

CoCHG Confidence that the site is better than moderate class.

CoCMPB Confidence that the site is moderate or worse class.

RoM G/M Rick of misclassification above / below good / moderate

boundary.

	Site code	No. Samples	No. missing	Mean EQR	Class	CoC High	CoC Good	CoC Mod	CoC Poor	CoC Bad	RoM	CoC HG	CoC MPB	RoM-G/M
1	36082	2.00	0.00	0.82	High	57.27	39.44	3.23	0.07	0.00	42.73	96.71	3.29	3.29
2	34649	2.00	0.00	0.81	High	53.00	43.23	3.69	0.07	0.00	47.00	96.23	3.77	3.77
3	36073	2.00	0.00	1.29	High	100.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
4	35965	2.00	0.00	1.22	High	100.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
5	35101	1.00	0.00	0.47	Moderate	0.04	12.07	62.00	25.68	0.22	38.00	12.10	87.90	12.10
6	35075	2.00	0.00	0.71	Good	15.38	71.06	13.34	0.22	0.00	28.94	86.44	13.56	13.56
7	36222	2.00	0.00	0.52	Moderate	0.00	15.25	76.77	7.98	0.00	23.23	15.25	84.75	15.25
8	36203	2.00	0.00	0.82	High	60.23	36.77	2.93	0.06	0.00	39.77	97.00	3.00	3.00
9	36207	1.00	0.00	0.41	Moderate	0.00	3.44	50.89	44.93	0.73	49.11	3.44	96.56	3.44
10	35476	2.00	0.00	0.84	High	64.73	32.68	2.53	0.06	0.00	35.27	97.41	2.59	2.59
11	34671	2.00	0.00	0.59	Moderate	0.35	47.06	50.84	1.75	0.00	49.16	47.41	52.59	47.41
12	35962	1.00	0.00	0.79	Good	45.68	42.09	10.90	1.31	0.02	57.91	87.77	12.23	12.23
13	33222	1.00	0.00	0.54	Moderate	0.56	29.65	57.29	12.42	0.08	42.71	30.21	69.79	30.21
14	35557	1.00	0.00	1.31	High	100.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
15	122818	2.00	0.00	0.63	Good	1.56	61.11	36.45	0.88	0.00	38.89	62.67	37.33	37.33
16	122740	2.00	0.00	1.10	High	100.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
17	122741	2.00	0.00	1.15	High	100.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
18	122459	2.00	0.00	0.80	Good	49.32	46.46	4.14	0.08	0.00	53.54	95.78	4.22	4.22
19	122463	2.00	0.00	0.97	High	98.06	1.82	0.11	0.01	0.00	1.94	99.88	0.12	0.12
20	122682	2.00	0.00	0.81	High	54.12	42.24	3.57	0.07	0.00	45.88	96.36	3.64	3.64
21	122827	2.00	0.00	0.77	Good	36.14	57.48	6.27	0.10	0.00	42.52	93.62	6.38	6.38
22	122856	2.00	0.00	1.32	High	100.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
23	122857	2.00	0.00	1.00	High	100.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
24	122858	2.00	0.00	0.69	Good	8.79	72.22	18.67	0.32	0.00	27.78	81.01	18.99	18.99
25	122824	2.00	0.00	0.70	Good	12.06	72.08	15.60	0.26	0.00	27.92	84.14	15.86	15.86
26	122638	2.00	0.00	0.99	High	99.85	0.15	0.00	0.00	0.00	0.15	100.00	0.00	0.00
27	122675	2.00	0.00	1.08	High	100.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
28	122678	2.00	0.00	0.60	Good	0.49	50.20	47.79	1.52	0.00	49.80	50.70	49.30	49.30
29	122679	2.00	0.00	0.57	Moderate	0.08	34.26	62.58	3.08	0.00	37.42	34.34	65.66	34.34
30	122832	2.00	0.00	0.61	Good	0.75	54.29	43.71	1.25	0.00	45.71	55.04	44.96	44.96
31	122835	2.00	0.00	1.05	High	100.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
32	122860	1.00	0.00	0.44	Moderate	0.01	6.67	58.03	34.88	0.40	41.97	6.68	93.32	6.68
22 1 4   +  \S	1441	nmary $\lambda$ Unce	0.00	0.04	High	01.02	7.24	0.70	0.07	0.00	0 N7	00.44	N 00	N 00

Figure 7: Uncertainty analysis output

# 5. Viewing the original data

Figure 8 shows the listing from the "view data" sheet. Taxa either not matched to the master taxon list or matched but lacking TDI scores are highlighted in red. In the example shown planktonic taxa are highlighted as not-matched. This option can be useful in revealing misidentifications, mis-codings or problematic samples with high abundances of taxa not currently in the database.

🔯 Da	Data view: DARESTest.xls											
	А	В	С	D	E	F	G	Н	- 1	J	^	
1	SampleID		SPR001	AUT001	SPR002	AUT002	SPR003	AUT003	SPR004	AUT004	SPR0	
2	SiteID		36082	36082	34649	34649	36073	36073	35965	35965	3510·	
12	ZZZ912	Achnanthidium biasolettiana	0.00	0.00	0.00	0.00	2.15	5.40	1.27	0.31	0.00	
13	AD009A	Achnanthidium microcephalum	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
14	ZZZ835	Achnanthidium minutissimum type	16.93	1.26	7.31	2.11	27.30	37.14	13.97	11.38	13.86	
15	ZZZ911	Achnanthidium subatomus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
16	AP001A	Amphipleura pellucida	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	
17	AM013A	Amphora inariensis	1.25	0.00	1.33	0.00	0.00	0.00	0.00	0.00	0.32	
18	AM011A	Amphora libyca	1.57	0.00	0.66	0.00	0.00	0.00	0.32	0.00	0.00	
19	AM084A	Amphora montana	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
20	AM001A	Amphora ovalis var. ovalis	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	
21	AM012A	Amphora pediculus	3.76	0.00	5.32	3.17	3.37	1.90	3.81	2.77	35.02	
22	AM9999	Amphora sp.	0.00	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00	
23	AM004A	Amphora veneta var. veneta	0.00	0.00	0.00	0.00	0.00	0.32	0.00	0.00	0.00	
24	AS001A	Asterionella formosa var. formosa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
25	AS003A	Asterionella ralfsii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
26	AU003A	Aulacoseira granulata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
27	AU9999	Aulacoseira sp.	0.00	0.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
28	BR9999	Brachysira sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
29	BR001A	Brachysira vitrea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
30	CA002A	Caloneis bacillum var. bacillum	0.00	0.31	0.00	1.06	0.00	0.00	0.00	0.00	0.00	
31	CA9999	Caloneis sp.	0.00	0.00	0.66	0.00	0.00	0.00	0.00	0.00	0.00	
32	CV001A	Cavinula cocconeiformis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
33	CV005A	Cavinula variostriata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
34	UN9995	Centric undif.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
35	CO010A	Cocconeis disculus	0.00	0.00	0.33	0.00	0.31	0.00	0.00	0.00	0.00	
RE (	Iconnsa III	Cocconeis nediculus	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	n nn 1	

Figure 8: Data view

# 6. Saving the results

Results can be saved to an Excel file by clicking the Save button or selecting File -> Save results from the main menu. Results are saved in a series of worksheets with the same layout and column headings as listed in the "View Results" sheets and described above.

# 7. Acknowledgements

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#### 8. References

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