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Cryptosporidium and *Giardia* in Drinking Water: Discussion document for selection and evaluation of an NMKL Method

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CONTENTS

Executive Summary

The objective of this discussion document is to reach a consensus on the most appropriate approach to take regarding the description of an NMKL method for analysis of water for *Cryptosporidium* and *Giardia*, by consideration of approaches in place elsewhere in the world.

In UK and USA, validated and legislated methods are in place. In other countries, no particular methods are recommended or legislated. However, for all countries considered a period of data gathering for risk assessment is stipulated and the inclusion of relatively stringent QA controls.

In Nordic countries, monitoring has largely been *ad hoc* or research-driven, and based on the method from USA. However QA procedures are generally inadequate and recovery efficiencies fluctuate greatly.

It is proposed that implementation of a proscribed NMKL method now is inappropriate, and adoption of one of the validated and legislated methods from elsewhere is probably inappropriate and could be restrictive

However, implementation of guidelines would be recommended and stipulated inclusion of QA procedures.

Summary

In this discussion document the current situation elsewhere in the world regarding standard techniques for analysis of water for the parasites *Cryptosporidium* and *Giardia* are described, and compared with the present situation in Nordic countries. The objective is to reach a consensus on the most appropriate approach to take regarding the description of an NMKL method for analysis of water for *Cryptosporidium* and *Giardia*.

In UK and USA, validated and legislated methods are in place, and both are based upon 4 steps of 1) filtration; 2) elution and clarification; 3) separation; and 4) detection. However, there are various differences between the methods validated and equipment/reagents stipulated between US and UK. In other countries, such as Australia, Canada and New Zealand, no particular methods are recommended or legislated, and also routine monitoring is generally not recommended, but usually event-

based monitoring (e.g. following heavy rainfall). However, for all countries in which consideration of methods and monitoring is documented, a period of data gathering for risk assessment is stipulated and the inclusion of relatively stringent QA controls.

In Nordic countries, where monitoring has largely been *ad hoc*, or research-driven, the methods are broadly based on those from USA, although with less attention to some of the detail of the methodologies, and QA procedures are generally inadequate. Where recovery efficiencies have been measured, these fluctuate greatly between individual laboratories/countries, ranging from 10 to 85%.

It is proposed that at this time, the implementation of a proscribed NMKL method is inappropriate, and adoption of one of the validated and legislated methods in place elsewhere could also be restrictive, and is probably inappropriate for the current situation in Nordic countries. However, implementation of guidelines would be recommended, based on the 4 steps listed above, and with stipulated inclusion of QA procedures, based on those described in the US EPA method.

Purpose of Document

In August 2004, the Norwegian National Committee in the Nordic Committee on Food Analysis (NMKL) decided that a standard NMKL method for the detection of the parasites *Cryptosporidium* and *Giardia* in water should be prepared. A referent (Lucy Robertson, Norway) was accordingly appointed, and, over the course of the next 10 months, appropriate contact persons in each of the participating countries were also appointed as listed below.

Denmark: Linda Bagge from the Danish Environmental Protection Agency (replacement for Vibeke From Jeppesen from Chr. Hansen A/S) and Heidi Enemark (to be replaced by Charlotte Maddox-Hyttel) from the Danish Veterinary Laboratory.

Finland: Ruska Rimhanen-Finne from the Department of Food and Environmental Hygiene at Helsinki University, Finland.

Iceland: Sigurður Richter from the Institute for Experimental Pathology at the University of Iceland.

Sweden: Anette Hansen from the Smittskyddsinstitut in Sweden

As with some other microbiological methods, but probably more than most, the analysis of water for parasites has evolved considerably over the past 10 years, with the introduction of superior filtration devices, designed specifically for parasites, and also with the development of immunomagnetic separation (IMS) techniques which have greatly improved the separation possibilities over the traditional flotation or sedimentation techniques. Although molecular detection techniques for these parasites are available, they are presently used more as a research tool than for routine analysis.

The purpose of this discussion document is to summarize the current situation elsewhere in the world regarding standard techniques currently validated for analysis of water for these parasites, and compare this with the present situation in Nordic countries. On the basis of this comparison and associated discussion it is hoped that a consensus should be reached on the most appropriate approach to take regarding the description of an NMKL method.

Background

The possibility of waterborne transmission of giardiasis and cryptosporidiosis is well recognised, and several communitywide waterborne outbreaks of both infections have been documented worldwide. In some of the outbreaks large numbers of individuals, several hundreds, or even thousands, have been considered to have been affected.

Various factors contribute to the potential for waterborne transmission of *Cryptosporidium* and *Giardia*. These include:

- low host specificity (infect a wide range of animals as well as man)
- robust transmission stages means the parasites can survive traditional disinfectant regimes (e.g. chlorination) used by water industry
- small size of transmission stages (allows penetration of standard drinking water treatment filters)
- high numbers of transmission stages excreted by infected hosts
- low infectious dose

Whilst most outbreaks have been documented predominantly from UK and USA, there have been a number of outbreaks reported in Scandinavia. In particular, there have been a number of waterborne outbreaks of giardiasis documented in Sweden, as well as

a swimming-pool associated outbreak of cryptosporidiosis, and a recent waterborne outbreak (Autumn/Winter 2004) of giardiasis in Bergen, Norway with over 1400 individuals diagnosed as infected. No waterborne outbreaks of either infection have been documented from Denmark, Finland or Iceland.

In many countries water sources and/or treated water are routinely monitored for *Cryptosporidium* and/or *Giardia*, and in some countries legislation has been implemented which mean that monitoring must be conducted following certain stipulated guidelines and/or following particular validated methods. The USA and UK are both countries in which legislation is in place with validated, proscribed methods, and it is pertinent for our purposes to consider, in overview, the approaches used in these places. This is not only to avoid repeating work or assessments already completed, but also because the approaches used in the two countries are to some extent substantially different, and the decision of which approach the Nordic countries considers most appropriate, or whether both approaches should be included, may be in part decided by the experiences obtained in these two different situations. The background and general information is outlined below, with further details of the methods used in each case given in subsequent sections.

Additionally the situation in Australia is considered, as this country too has expended considerable effort in deciding upon its approach for monitoring of water supplies for these parasites.

In USA, a large waterborne outbreak of cryptosporidiosis in Milwaukee in March/April 1993 in which 403000 were estimated to have been infected and significant mortality demonstrated, particularly in the immunocompromised, may have been to some extent a spur which hastened the implementation of various ‘rules’ and use of proscribed methods. In 1996 the ‘Information Collection Rule’, was introduced in which monitoring of source water (used by large public water systems) had to be conducted for various parameters, (including the occurrence and concentration of *Cryptosporidium* and *Giardia*), over an 18 month period to enable potential health risks to be assessed and to guide future regulatory and public health decisions. In 1999 US Environmental Protection Agency (EPA) Methods 1622 and 1623 were introduced, describing specific methods for the analysis of water for these parasites. Based on the information collected as part of the ICR and also an initial (introduced in 1989) ‘Surface Water Treatment Rule’ (SWTR) further treatment rules have been, and are being, introduced, including the Interim Enhanced Surface Water Treatment Rule (IESWTR), the Long Term 1 Enhanced

Surface Water Treatment Rule (LT1ESWTR) and the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). These latter rules, which particularly address *Cryptosporidium* (which was not addressed in the original SWTR) include rules for treatments to remove (or inactivate) *Cryptosporidium* oocysts by designated log removals, with additional treatments or barriers to be included which are dependent upon the classification of a particular water source. The classification is based upon monitoring using Methods 1622 and/or 1623 (both of which were up-dated in 2001) with samples collected on at least a monthly basis. The information may have, in part, been collected as part of the ICR, part of the ICR supplemental surveys (ICRSS) conducted between March 1999 and February 2000, or subsequently.

In UK, an outbreak of waterborne cryptosporidiosis occurred in 1995, in which 575 individuals were infected. Due to the private ownership of water supplies in UK, the company owning the implicated water supply was prosecuted in court, but the evidence was considered inadmissible. Apparently as a direct result of this failed prosecution, the UK Drinking Water Inspectorate (DWI) introduced risk assessment and monitoring legislation in 1999 (The water supply (water quality) (amendment) regulations 1999, SI 1524). As part of these regulations each water treatment plant in England and Wales had to conduct a risk assessment to establish whether there was a significant risk from *Cryptosporidium* oocysts in the water supplied from the particular works, following a devised protocol and including an extensive list (56 possible factors, divided into 7 groups) of possible risk factors. In treatment plants in which it was considered that there was a significant risk, either equipment had to be installed which was considered to be capable of removing particles of greater than 1 µm diameter, or a continuous, on-line monitoring of the treated water for this parasite had to be installed. The monitoring regime was published as a Standard Operating Protocol in 4 parts (Sampling and Transportation of Samples; Laboratory and Analytical Procedures; Validation of New Methods or Parts of Methods for Sampling and Analysis; Requirements for the Inter-Laboratory Proficiency Schemes).

In Australia, during July to September 1998, high numbers of both *Cryptosporidium* oocysts and *Giardia* cysts were detected in the water supply for Sydney (both in treated and untreated water), and ‘boil water’ notices were issued for 3 million residents during this period. Although no increase in human (or animal) infection was noted during this period, despite increased epidemiological surveillance, the incident was widely reported around the world (possibly partly because the Olympic Games were scheduled to take

place in Sydney the following summer), and a government inquiry into the incident was commissioned in which one of the recommendations was for evaluation of whether water monitoring by a validated methodology should be implemented. As a result of this, the Australian Drinking Water Guidelines (ADWG; as developed and up-dated by the Australian National Health and Medical Research Council in collaboration with the Australian Natural Resource Management Ministerial Council) now contain sections on both these parasites (http://www.nhmrc.gov.au/publications/_files/awg5.pdf. For both parasites catchment control (minimizing contamination of source water) and multi-barrier water treatment is recommended, but no routine monitoring. Instead investigative and event-based monitoring of source waters is recommended, with events such as heavy rainfall, treatment failure, and increase in disease incidence in the community, suggested as potential triggers for investigation. Additionally, the ADWG state that "Sanitary surveys of water catchments should be undertaken". This is in order to assess risk factors for contamination and provide a basis for catchment management to reduce these risks.

In Sydney, where the contamination event occurred, two years of worth of data were collected from 1998 onward, and as a result of that, a programme has been initiated which involves treated water being monitored at 3 locations 6 days/week (100 litre sample size), event monitoring at sites leading into a lake (events being storms and other factors which may cause stream levels to rise or influence water quality), monitoring at spots considered to be at elevated risk of contamination, and monitoring of other parameters which may be indicators of contamination. Other water storage areas are analysed on a weekly basis.

However, the ADWG do not recommend a particular methodology for analysis of water as they state that "At this time, there is insufficient information to prescribe a standard method for testing water for the presence of *Cryptosporidium*/*Giardia*." However, the ADWG do state that "The use of any method should incorporate exacting quality control procedures and include determination of recovery efficiencies." In Sydney the analysis is conducted by a laboratory which has been accredited by the Australian National Association of Testing Authorities.

The Guidelines for Canadian Drinking Water Quality (http://www.hc-sc.gc.ca/hecs-sesc/water/pdf/summary.pdf), and Drinking Water Standards for New Zealand (http://www.moh.govt.nz/moh.nsf) are apparently similar to those from Australia.

USA (EPA) Method

The protocol for assessment of water supplies for *Cryptosporidium* and *Giardia* in USA is based on the requirement to analyse relatively small (ca. 10 L) volumes of raw, untreated, source water. The protocols, EPA Method 1622 (for *Cryptosporidium* only) and 1623 (for *Cryptosporidium* and *Giardia* together), were first published in 1999, and up-dated in 2001 are available on-line (http://www.epa.gov/nerlcwww/1622ap01.pdf and http://www.epa.gov/nerlcwww/1623ap01.pdf). The methods were up-dated to allow the inclusion of various new equipments and reagents which had not previously been available when the first edition was prepared. In brief, the procedure consists of 4 defined steps, as follows:

1) the water sample is filtered,
2) the materials captured on the filter eluted and concentrated
3) the *Cryptosporidium* oocysts and *Giardia* cysts separated by immunomagnetic separation (IMS)
4) the separated oocysts and cysts are dried and fixed onto microscope slides, which are then stained with fluorescent reagents and screened by fluorescent microscopy.

Approved products have been validated for use at each stage in the method. According to the EPA, other options can be used provided that their acceptability is demonstrated by fulfilment of described procedures. For the filtration step, three filtration set ups have been suggested for use, namely: a) Envirocheck™ Sampling Capsule (Pall Gelman Laboratory); b) CrypTest Capsule Filter (Whatman Inc.); and c) Filta-Max™ (IDEXX).

Filtration: Filtration can be done *in situ* at the water sampling site, or in the laboratory following transport to the laboratory of the sample. When the first draft of EPA Method 1622 was first printed in 1997, membrane filtration was also included as a filtration method. However, it was not included in the final version (apparently due to less ease of use). Nevertheless, membrane filtration continues to be used relatively widely, particularly in research settings, especially it is less expensive than the other options available. With regards to Nordic countries, membrane filtration is at present in use at the Parasitology Laboratory at NVH, Oslo, Norway, SMI, Stockholm, Sweden and Helsinki University, Finland. A summary of the filtration options currently used in Nordic countries is described in table 1, below.

Table 1: Filtration options in analysis of water for *Cryptosporidium* and/or *Giardia* as presently used in Nordic countries.

Filtration option	Used by:
Membrane filtration	NVH, Norway; SMI, Sweden; HU, Finland;
Envirocheck Sampling Capsule	M-Lab, Norway; HU, Finland
Filta-Max	Available for use at NVH, Norway, but not currently operational.

Elution/concentration: The precise method of elution of the materials from the filter is dependent upon the type of filter used, but basically consists of washing in a detergent buffer, with concentration of the eluate by centrifugation, and if Filta-Max filtration has been used, a small membrane filtration step. Concentration of the eluate from the Envirocheck Sampling Capsule is also done by small membrane filtration in Finland.

Separation: IMS procedures follow the manufacturer's instructions. For Method 1622 and 1623, only the IMS kits made and sold by Dynal Biotech ASA are listed in the procedure, and are, indeed, the only ones used in water analysis in Nordic countries at this time (??).

Detection: Three different fluorescent monoclonal antibodies (Mab) have been validated for use in Method 1622/1623, three made by Waterborne Inc (New Orleans, USA), one for each parasite, and one combined Mab, and one made by Meridian Diagnostics (Cincinatti, USA). In Nordic countries, the following monoclonal antibodies are used routinely by laboratories analysing water samples for parasites:

- Aqua-Glo, Waterborne Inc. (used by NVH, Norway; M-lab, Norway; HU, Finland; SMI, Sweden)

UK (DWI) Method

The protocol for assessment of water supplies for parasites in UK (England and Wales) is based on the legal requirement for constant analysis (daily 24 h samples) of treated water for *Cryptosporidium* oocysts, as it leaves the treatment works, in works which having undertaken a risk assessment are considered to be at risk of contamination of by *Cryptosporidium* oocysts.

The standard operating protocols (SOPs), which were first published in 2000, have amendments added at regular intervals as separate amendment documents. All the documents are available on-line (http://www.dwi.gov.uk/regs/crypto/legalindex.htm). As with the US methods, these documents are largely up-dated to allow the inclusion of

various new equipments and reagents which had not previously been available when the first edition was prepared. Rather than being published as a single document, the SOPs have been prepared as a series of documents in 4 major sections as follows (Sampling and transportation of samples; Laboratory and analytical procedures, with a separate appendix for Laboratory quality assurance tests; validation of new methods, or parts of methods, for sampling and analysis; requirement for inter-laboratory proficiency schemes). The risk assessment, regulations, and amendments, as well as other details, are all published as separate documents. In practice the separation of the documents, and the inclusion of new methods as separate documents does not make for user friendliness. This is particularly so for the amendments, where, for example, for the laboratory and analytical procedures SOP, there have been 10 separate amendments added in under less than 2 years, but the main documents are also completely revised regularly. For the validation section, a completely revised document was prepared in January 2005, and for the laboratory and analytical procedures a completely revised document was prepared in May 2005.

Presumably because of the background to the introduction to this protocol, the methods are described so that if they are followed rigorously the conditions would be suitable to 'permit the use of the analytical results as evidence in a Court of Law'. Thus the system described gives a chain of evidence which is 'in compliance with the Police and Criminal Act, 1984'.

However, in brief, the procedure consists of the same 4 defined steps as used in the USA (EPA) method, as follows:

1) the water sample is filtered,
2) the materials captured on the filter eluted and concentrated
3) the *Cryptosporidium* oocysts and *Giardia* cysts separated by immunomagnetic separation (IMS)
4) the separated oocysts and cysts are dried and fixed onto microscope slides, which are then stained with fluorescent reagents and screened by fluorescent microscopy.

An alternative to steps 3 and 4, is a modified IMS (using the Dynal kit and reagents but a modified method as described in the DWI protocol), the oocysts are captured onto a slide, labelled and then detected by a solid-phase cytometer (ChemScan RDI) before subsequent confirmation by microscopic examination of the slide. Currently this method

is not known to be used by laboratories analysing water samples for parasites in Nordic countries.

Approved products have been validated for use at each stage in the method. According to the DWI, other options can be used provided that their acceptability is demonstrated by fulfilment of described procedures (which are described in detail in the validation section of the SOPs).

Filtration: For the filtration step, the sample must be filtered *in situ* at the water works. The rate of sampling is set to be a minimum of 40 L per hour, so that in 24 hours the minimum volume filtered will be 960 L. With the US EPA method, filtration can be done *in situ* or in the laboratory, as the required volume for filtration is much lower. The following filtration set ups have been validated for use, namely: a) Filta-Max™ (IDEXX); and b) Envirocheck™ HV filter (Pall Gelman Laboratory).

Elution/concentration: The method of elution of the materials from the filter is dependent upon the type of filter used, but basically consists of washing in a detergent buffer, with concentration of the eluate by centrifugation, or by membrane filtration and centrifugation if Filta-Max filtration is used.

Separation: IMS procedures basically follow the manufacturer's instructions. Those which have been validated for use in UK include the Dynal Biotech IMS, and TCS Bioscience/ ImmuCell Crypto IMS (Isolate Cryptosporidium). As the UK method is only aimed at analysis for *Cryptosporidium*, and not *Giardia*, the only IMS systems validated are those aimed at separation of *Cryptosporidium*. TCS Bioscience/ImmuCell does not presently have an IMS system for isolation of *Giardia*. The IMS options currently used in Nordic countries are described in table 2, below.

Table 2: Immunomagnetic separation options in analysis of water for *Cryptosporidium* and/or *Giardia* as presently used in Nordic countries.

IMS option	**Used by:**
Dynal Biotech (anti-Cryptosporidium; GC-combo)	NVH, Norway; SMI, Sweden; HU, Finland; M-lab, Norway;
ImmuCell/TCS Bioscience (Isolate Cryptosporidium)	
Aureon (Giardia, Crypto, Crypto-Giardia combination)	NVH, Norway (only rarely used)

Detection: Three different fluorescent monoclonal antibodies (Mab) have been validated for use in the UK (DWI) method. These are Cellabs Cryptocel IF antibody (Cellabs), Microgen FITC-conjugated anti-Cryptosporidium antibody (Microgen Bio-Products) and

EasyStain (Biotech Frontiers). Currently none of these are known to be used by laboratories analysing water samples for parasites in Nordic countries.

Because of the way the method validation system is in place in UK, in which if part of the method is changed, the whole method must be tested with each equipment combination, not all of the filtration apparatus is currently validated with both the IMS systems nor with all the Mab. Similarly, both IMS systems are not currently validated with all the Mab. This also means that although the protocols are regularly up-dated, there may be some lag before combinations which have been validated are published as approved.
In Scotland and in Northern Ireland, similar laboratory procedures are followed as in England and Wales, although the legislation involved with their implementation is separate (http://www.dwqr.org.uk/documents.html and http://www.ehsni.gov.uk/pubs/publications/Crypto_Guidance_feb03.pdf) .
In Ireland (Eire), whilst the issue of monitoring water supplies for parasites (specifically Cryptosporidium) has been addressed, and risk assessment and appropriate monitoring schemes recommended, there has apparently been, as yet, no stipulation of methodology which should be used (http://www.ndsc.ie/Publications/ConsultationDocuments/d449.PDF).

Quality assurance/laboratory proficiency schemes

Both the US EPA protocol and the UK DWI protocol include a section on quality assurance, and this is obviously important for ensuring confidence in results obtained. For the UK the QA is designed such that any results obtained are admissible as evidence in a Court of Law.
US EPA protocol: In the US EPA method the minimum requirements to be fulfilled by a laboratory are initial demonstration of laboratory capability by performance of an IPR (initial precision and recovery) test in which a spike at a known concentration (100-500 cysts/oocysts) are used in 4 samples of reagent water and recovery and relative standard deviation (RSD) are calculated. The results must be within certain defined limits for the laboratory to be considered capable of conducting water analysis for these parasites. Additionally analysis of spiked samples (in matrix water as well as reagent grade water) are required to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance (OPR = ongoing precision and

recovery). The OPR testing (analysis of 1 spiked reagent water sample) must be conducted on a weekly basis, or if more than 20 samples are analysed per week, one OPR test for every 20 samples. Additionally a blank (negative sample) must be analysed on the same time schedule. For the spiking studies it is recommended (although not mandatory) that flow-cytometer sorted organisms are used, either prepared internally or bought.

Periodic (at least monthly) enumeration of internally prepared slides (40-100 cysts and oocysts, of which approximately 50% are DAPI positive) and comparison between analysts in a single laboratory is also stipulated.

Periodic analysis (no designated period) of an external QC sample (performance evaluation sample or a reference sample) is also stipulated, as well as periodic participation in inter-laboratory comparison studies.

Other laboratory QA requirements are annual calibration of micropipettes, microscope certification and servicing and proper auditable record-keeping. For every batch of slides screened a positive and negative control must also be included.

If a laboratory wishes to modify the method protocol from that described, then IPR testing must be documented which demonstrates equivalence to the protocol, and if it is intended that the modification is included in the protocol for nationwide use, then similar tests must be performed in at least 3 different laboratories. However, modifications to the protocol are only permitted for the first 3 stages. Although modification to the immunofluorescence detection method is allowable, substitution with another method of detection is not allowed.

US DWI protocol: In the UK DWI protocol the QA stringency is based on the intention that any results obtained are admissible as evidence in a Court of Law. Therefore, every laboratory in UK (England and Wales) analysing water samples for *Cryptosporidium* must also have an in-house ‘sampling rig’ by which a spiked sample can be prepared. A spiked sample must be prepared and analysed every day that a ‘real’ sample is analysed and the results recorded and plotted graphically, following a specifically designed system in which ‘warning’ and ‘response’ parameters are included. The spike must be flow cytometer prepared, and be of 100 oocysts, and prepared either in-house (following approved procedures) or purchased from approved suppliers.

Participation in an approved and DWI moderated inter-laboratory proficiency test on a monthly basis is mandatory. The proficiency test includes microscope slides, suspensions and filters (a minimum of 1 of each of these analysed each month).

Other laboratory QA requirements are extensive, and laboratories which undertake these analyses must be approved by DWI. Other stipulations include the set up and design of the laboratory, training of staff, colour-blindness testing of microscopists, and detailed documentation trails. Annual audits, and unannounced audits, are also stipulated. For every batch of slides screened a positive and negative control must also be included.

If a laboratory wishes to modify the method protocol from that described, then a full laboratory appraisal is required, the results of which must be submitted to the DWI in writing, and must be approved before implementation is allowed. The appraisal process requires both single laboratory and multi-laboratory validation to ensure equivalent or enhanced performance from the proposed modifications.

Other method options not included in either protocol

The main alternative option which is not included in either the UK DWI or USA EPA protocol is the use of alternative methods for detection. These methods include molecular methods (e.g. PCR), sorting methods (e.g. flow cytometry), use of microwell array chips (using oligonucleotides or immobilised antibodies). Whilst a number of publications have cited these methods as having equivalent or superior sensitivity and reproducibility to microscopy, they have not been included in either the UK DWI or US EPA protocols. Whilst such methods have a number of advantages (speed, possibility of simultaneous genotyping for molecular methods, combine with viability assessment), there are also a number of disadvantages including equipment costs and maintenance costs and the inability to detect non-nucleated organisms.

The situation in Nordic countries at present; recovery efficiencies/numbers of samples analysed

Monitoring of water for *Cryptosporidium* and *Giardia* in Nordic countries varies markedly between countries. Sweden, from where one of the first European waterborne outbreaks of giardiasis is recorded, has the longest history of analysing water for these parasites, whereas in Iceland there is currently no monitoring undertaken and no laboratories presently have the necessary equipment or experience. In Norway, the first water analyses of *Cryptosporidium* and *Giardia* were undertaken in the 1990s, and there is a small amount of routine monitoring of water sources and treated water for these

parasites. On the whole, however, such monitoring is limited and sporadic. Denmark and Finland commenced some water analyses for these parasites shortly after Norway, but on the whole these analyses are undertaken for research reasons, rather than as routine monitoring programmes. In general the methods which are in use are more similar to the US EPA protocols, although with some variations from the detailed method. Membrane filtration (not mentioned in the final US EPA protocol, although considered in the original drafts) is commonly used, and there are some deviations from the methods in terms of how frequently reagents are prepared, and time gaps between different stages of the procedure. As such analyses have generally been undertaken for research, it is unsurprising that various of the requirements stipulated in the protocols have not been followed with strict attention to detail.

Notably, however, the requirement for on-going and regular analysis of spiked samples for continuous validation of the method and laboratory performance is largely ignored, as is the participation in external QA schemes. Comparison of method details between the countries are listed in tables 1-2 previously. In table 3 below, a summary of further details regarding the current situation, and anticipated changes, in each of the Nordic countries are outlined for comparative purposes, and includes the present implementation of Quality Control. This is generally poor, varying between ‘sporadic’ to ‘annual’ to considerably less than annual.

Although it is widely acknowledged that the methodologies available for analysis of water samples for these parasites are highly variable in efficiency, and that this variation is also influenced by water quality, the variation in reported recovery efficiencies (from approximately 10% in Finland up to 60-85% in Sweden) is concerning. This suggests that IPR (as detailed in the US EPA methodology) should be implemented as a matter of some urgency and inter-laboratory comparisons and trials should be considered, as well as participation in external QA schemes (currently only done by SMI, Sweden).

Table 3: Current situation regarding analysis of water for *Cryptosporidium* and/or *Giardia*: comparison between Nordic countries.

	Denmark[1]	Finland	Iceland	Norway		Sweden
How many labs analyse water for *Cryptosporidium* and *Giardia*		1	0	2		1
				NVH	M-lab	
Approximately how many samples per year?		30	NA[2]	Ca. 50	Ca.35	Ca. 100
What proportion of these samples are routine monitoring and what proportion are for research?		Mostly research	NA	In previous years, mostly research. In 2004, 20 research, 24 monitoring.	All routine monitoring	Variable – both research and routine monitoring.
Is this likely to change in the foreseeable future?		No	It is intended that some initial monitoring will be conducted, with analyses conducted outside of Iceland	Yes, slight increase due to Bergen outbreak	Yes, increase anticipated due to new Mattilsynet regulations for parasites	Unknown; possibly likely due to Bergen outbreak.
How often are spiked controls run in reagent water?		Spiked controls are only run in matrix water.	NA	At least annually.	Approx. annually	Spiked controls are only run in matrix water.
How often are spiked controls run in matrix water?		Twice in 4 years.	NA	Sporadically – (for example, for a new project)	Sometimes run in tap water	Sporadically (for example, at the beginning of a new project)
Are the spikes made in lab (flow cytometer sorted or dilutions of stock suspensions counted by microscopy) or purchased?		Dilutions of stock, counted by microscopy	NA	Dilutions of stock, counted by microscopy	Dilutions of stock, counted by microscopy	Purchased: Colour-seed (Biotech Frontiers)
What are the recovery efficiencies?		Approx. 10%	NA	Approx. 45% for Crypto, and approx. 65% for Giardia.	Approx. 27%	Approx. 60-85%
Is there participation in any external QC schemes?		No	NA	No	No	Yes, HPA Quality Control Scheme.

1. Data from Denmark will be included as soon as possible.
2. NA=not applicable

Discussion: exploiting experiences from elsewhere and transferring to the Nordic situation

It is pertinent for the Nordic situation, to consider the situations for analysis of water for *Cryptosporidium* and *Giardia* elsewhere in the world, particularly USA, UK and Australia. In all these countries, a substantial part of the drive for providing a guideline or a recommended or prescribed method appears to have occurred because of the occurrence of particular contamination events which have impacted on the water-consuming public, due to disease outbreak and/or because of the necessity for a prolonged 'boil water' notice being served. Whereas both UK and USA have detailed and validated methodologies in place which must be followed (the UK method can perhaps be considered as the more stringent of the two, as it is designed to provide evidence which can be used in a Court of Law), in Australia neither routine monitoring, nor a numerical guideline, nor a prescribed method has been mandated.

For all 3 countries, some sort of initial 'risk assessment' of catchments or water sources is included in the methods or guidelines, in order both to enable the risk of contamination to be minimised, and also in order to assess the likelihood of contamination events occurring, and the degree of removal or inactivation likely to be required. Whilst various mostly '*ad hoc'* studies have been conducted in Nordic countries, notably in Sweden, but also to a lesser extent in Norway, Finland and Denmark, there has apparently been no systematic risk assessment of water sources and water catchments put in place, and this would seem to be a pertinent approach to take.

In Nordic countries there has been very little apparent requirement for routine monitoring, but background information indicates that these parasites are relatively widely spread in both human and animal populations and in the environment. As such any investigation of water sources for these parasites has on the whole been research-driven, and therefore the technique employed has often put cost considerations ahead of considerations such as ease of use, which is presumably the reason why membrane filtration is the most frequently used filtration technique in Scandinavia. Although this may not be the 'best' method in terms of ease of use, as long as the recovery efficiency and reproducibility are satisfactory, there seems to be no pressing requirement for stipulating an alternative filtration method. One disadvantage of membrane filtration is the requirement for transporting a relatively large volume of water (10L) from the water source to the laboratory. However, in most instances this should not be too burdensome.

During the outbreak of waterborne giardiasis in Bergen in 2004, numerous 10L water samples were sent from Bergen to Stockholm with apparently no difficulty.

The Nordic situation does not favour one particular methodological approach over another, and whilst the four steps of 1)filtration, 2) elution and concentration, 3) separation and 4) microscopic detection provide a basis, there seems to be no particular requirement or reason to favour, for example, one particular filtration technique over another, at the present time.

Adequate quality control is an integral part of the analytical method which is emphasised by the guidelines and/or legislation from UK, USA and Australia. In Nordic countries, the quality control in place would currently seem to be insufficient. Only the SMI in Sweden participates in an external quality control scheme, but internal quality control at all the laboratories in Nordic countries could be improved. The discrepancies in recovery efficiencies between the different laboratories are also of concern. Whilst the internal quality control procedures described in the UK DWI method are probably excessive for the Nordic situation, the situation of spiked samples being analysed only sporadically, annually or even less frequently is also not satisfactory, and an improved internal and inter-laboratory quality control scheme in and between Nordic countries should be implemented, with perhaps compulsory participation, particularly for laboratories which analyse water samples on a commercial basis rather than purely for research (i.e. are requested and paid for by external sources).

Conclusions

- This discussion document outlines the background to analysis of water samples for the parasites *Cryptosporidium* and *Giardia*, with particular emphasis on those countries which have legislated methodologies in place (USA and UK) and those which do not (Australia, Canada, New Zealand), and tries to relate this information to the situation in Nordic countries.
- Both the legislated methodologies from USA and UK are based on 4 sequential procedures (filtration, elution and concentration, separation and detection), but with significant differences stipulated between them.
- Currently in Nordic countries the procedures used in the laboratories undertaking these analyses follow these 4 steps, but without adhering to particular rules and

guidelines. This is probably partly because much of the analysis of water in Nordic countries at present is driven by research.

- As all the methods available are similarly inadequate and none is obviously superior to another, it would seem sensible that at present a detailed method is not stipulated, but that the four procedures listed in the 2nd bullet point above are followed.
- However, collection of catchment data for risk analysis, as described in the guidelines/legislation from UK, USA and Australia, is recommended for Nordic Countries.
- Additionally, improvement in QA is very necessary for laboratories undertaking these analyses in Nordic countries, and guidelines on such procedures could be formulated. The demonstration of laboratory capability by undertaking IPR testing (as described in US EPA method) would be a pertinent place to begin, with OPR testing, inter-laboratory trials and participation in an external QA scheme as additional recommended possibilities.
- Despite the known variability in recovery efficiencies associated with these methods, the large variation in recovery efficiencies reported from the different Nordic laboratories undertaking these analyses is concerning, and gives added weight to the requirement for internal IPR and OPR, as well as participation in external QA schemes.