Report from a survey of laboratory methods used in Scandinavia.

Testing for Lyme Borreliosis in the Nordic countries - variations in strategies and rate of seropositivity.

Ram Dessau[1], Ingvar Eliasson[2], Tone Skarpaas[3] og Dag Nyman[4]

[1]Department of Clinical Microbiology, Næstved Hospital, Region Zealand, Næstved, [2]Denmark; Department of Laboratory Medicine, NÄL, Trollhättan, Sweden; [3]Microbiology Unit, Division of Laboratory Medicine, Sørlandet Hospital HF, Kristiansand, Norway; [4]Department of Medicine, Åland Central Hospital, Mariehamn, Åland, Finland.

Contact address:
Ram Dessau, M.D., Ph.D.
Specialist in clinical microbiology
Department of clinical microbiology
Næstved Hospital, Region Zealand
61 Ringstedgade, DK4700 Næstved
Tel. +45 5651 4148
Fax. +45 5651 3717
Mail: ramd@regionsjaelland.dk

Abstract

Background. There is a running discussion both in the published literature and among the microbiology laboratories on the choice of strategy when testing for antibodies specific to *Borrelia burgdorferi* in clinical specimens. There are different commercial test kits on the market and external quality control schemes may show different results on the same sera using different methods.

Methods. A survey of laboratory methods used in Denmark, Finland, Norway and Sweden was performed. Forty-three laboratories responded and 35 also with production statistics, that is number of clinical samples tested and rate of seropositivity.

Results. Thirteen different methods were used for the primary ELISA or ELISA only. There were variations in the rate of seropositivity in the ELISA used on all samples from less than 5% to more than 60%. In the laboratories using a two-step strategy the re-classification rates varied from 12-88%. There were geographic differences in rates of seropositivity with a higher seroprevalence especially in Sweden. The two-tier principle was used in only 16 out of 43 laboratories and the selection criteria for two-tier analysis also varied. PCR was offered in six laboratories.

Conclusion. The variation in rates of seroposivity between the different assays and the re-classification rates for two-tier-testing reflected the lack of more specific recommendations for anti-borrelia antibody testing. Further development of recommendations for the laboratory diagnosis of Lyme borreliosis should be considered.

Introduction

Practical or sufficiently sensitive methods for detection of the causative agent: *Borrelia burgdorferi sensu lato* (Bb), culture or molecular tests for direct detection of antigen or DNA, have not yet been developed. The routine laboratory diagnosis of Lyme Borreliosis (LB) rests on detection of specific antibodies to Bb and not direct detection of the spirochete.
The diagnostic sensitivity is not optimal in the early stages of LB when duration of clinical disease is less than 4-6 weeks, due to late antibody response, especially in the case of isolated skin manifestations(1). The diagnostic specificity is low in endemic areas, mainly because of a high seroprevalence rate in the healthy population due to previous exposure with clinical or subclinical infection.
Thus a test with both high analytical sensitivity and high analytical specificity will have a lower diagnostic performance when used in endemic areas or in trying to confirm erythema migrans.
In many laboratories a large number of samples is tested each year. A number of these samples are requested also to rule out LB in patients with various unspecific symptoms. In this scenario a high positive background is a major problem. In addition, positive results, not related to active LB, may be caused by nonspecific cross-reactions. Guidelines recommend the use of immuno-blot (IB) to improve specificity(1,2). In the USA criteria for IB have been recommended. In Europe criteria have also been proposed (3), but no consensus has been reached and recommendations differ between countries whether to use just a sufficiently specific ELISA, two different ELISA's or to re-test (some of) the positive samples with an IB (two-step strategy) (4).
Commercial assays are marketed using different antigens or combinations of antigens. Which assay to choose is difficult as evaluations often rest on studies comparing the assays to each other. Even when the samples used for evaluation are selected on careful clinical criteria or laboratory criteria, misclassification cannot be ruled out. This has given problems for the external quality assessment schemes, where the same sample tested in different commercial assays has quite frequently given differences in results. As a gold standard is lacking it is however not known for certain which result is the more correct.
Ongoing discussions among microbiologists about these discrepancies motivated us to arrange a Scandinavian meeting concerning the laboratory diagnosis of Lyme Borreliosis (LB). The meeting was held in February 2009 (Örenäs Slott, Glumslöv, Sweden), with the objective to give an update on the laboratory diagnosis of LB in a Scandinavian context and to use the knowledge base of the participants in order to discuss guidelines and diagnostic criteria. (Scandinavia is in this article defined as Denmark, Finland, Norway and Sweden).

Preceding the conference, laboratories in Scandinavia were asked to supply data on the laboratory methods used. The results of this survey were presented at the meeting. The participants were mostly clinical microbiologists, but as the problems with Borrelia antibody testing are relevant to many clinicians, also in other countries we have chosen to publish the results of this survey. This report is intended as a contribution to the ongoing debate on laboratory diagnostics supporting the clinical diagnosis of LB.

Materials and methods

A survey was organized by the authors and a chart was sent to laboratories performing Borrelia diagnostics in each of the four countries. The questions were about methods used and crude production statistics with the number o-f positive results for the whole calendar year of 2008. Data were collected in January 2009.

Results

First line assays

Data from 43 laboratories were available for analysis. The response rate was 75% as 57 laboratories were known to perform Borrelia antibody testing (Table 1). Among the 43 different laboratories 9 different methods/combinations of methods were used for the test used on all samples received. Table 2 shows which commercial assays were used in 2008. 35 laboratories also provided production statistics.

The rates of seropositivity for nine different assays and four countries are shown (Figure 1). The graph is rather complex but the following patterns or tendencies may be appreciated:

1. The IDEIA flagella assay had a lower rate of seropositivity for IgG (2.2%-11.0%) compared to Enzygnost (9.2%-23.3%) and Liaison (10.9%-25.1%).
2. For IgM (green) the rates of seropositivity (9.4%, 3.7-19.5) were also quite variable, but appear less related to the assay.
3. The choice of assay preferred by the majority of laboratories for each country differered. For example Enzygnost was preferentially used in Norway, in Sweden both IDEIA and Liaison was used. In Denmark most laboratories used IDEIA.
4. Sweden had within each assay higher rates of seropositivity compared to Norway, with Denmark having the lowest rates. Possibly reflecting a higher endimicity of LB in Sweden.
5. The outlier (Finland position 3) was the highly endemic island of Aaland, where IgM is not analyzed.
6. Blood donor samples were typically data from approximately 100 samples so the rates have some stastistical uncertainty in the order of +/-5% as a 95% confidence interval to the binomial proportion. No blood donor data from the Enzygnost assay were available. IgM rates were uniformly low from 0% to 3% for all tests in the blood donors. Two Swedish laboratories using Liaison had high rates of seropositivity for IgG at 7% and 8% which could be due to high endemicity, as the rate of seropositivity in the routine samples was around 25%.

Two-step strategies

The strategies and choice of assay differ (Table 3). Twenty-seven laboratories used a one step strategy only. Sixteen laboratories used a two-step strategy. The selection criteria differed as some laboratories only retested samples from a few selected patients, others more systematically retested samples e.g. with low positive reactivity only. The rates of seropositivity of IgG in the second line test ranged from 12 to 86%, and ranging from 14% to 88% of samples were reclassified in the second assay. For IgM the reclassification rates range from 33 to 78 % in the second assay. The strategy on Åland was based on two ELISA's, C6 and Mikrogen IgG, as first line tests eventually followed by western blot if discordant results.

PCR

Six laboratories also offered PCR. Data was available from five laboratories: 2.4% of 582 spinal fluids were PCR positive and in joint fluids and skinbiopsies 13.1% were positive.

Intrathecal antibody production

The median rates of seropositivity for the measurement of intrathecal antibody production was 3.5 (range 0.9%-12.1%) for IgG and 3.9 (0.6% to 9.6%) for IgM using the IDEIA kit.

Thirty-three laboratories used IDEIA for detection of intrathecal antibody production. Only one laboratory used separate measurement of the permeability of the Blood brain barrier with the an adjusted index(5,6,6).

Discussion

Few studies have described rates of seropositivity in consecutive patients tested on the suspicion of LB. This study comparing the rates for seropositivity from many different laboratories all over Scandinavia show large differences (Figure 1). These differences seem to depend largely on the choice of assay (7). The choice of cut-off rather than the choice of antigen(s) or clinical epidemiology of LB could explain the differences related to assays. Seven laboratories provided test results from local blood donors. Healthy blood donors are not necessarily representative of a consecutive stream of symptomatic patients, but do probably express the lowest possible seroreactivity. These data may be used to make an assessment of positive predictive value PPV (Table 5). Let FPR be the number of false positive rate, TPR the true positive rate and TotPR the total positive rate. Assuming that TPR may be grossly estimated as TotP-FPR:

PPV = TPR/(TPR+FPR) = (TotPR-FPR)/TotPR

As the numbers of blood donors are relatively small the estimates have some statistic uncertainty so the estimates as such are not precise. The assays with higher rate of seropositivity in the consecutive routine samples also seem to have higher rates of seropositivity in the local healthy blood donors. The (TotPR-FPR)/TotPR is not clearly higher in the assays with high rates of seropositivity compared to IDEIA. Thus the old dogma: When increasing sensitivity the specificity becomes lower is confirmed. The number of true positives in the population is not known and these estimates are the probably lower as non-Lyme-Borrelia-patients with other diseases or other age-groups may have a higher frequency of cross reacting antibodies compared to healthy adult blood donors. These data were for practical reasons not adjusted for repeat samples from the same patients. In the experience from Næstved Denmark 2008 the rate of seropositivity for IgG is 2.9% using the first sample from each patient compared to 3.4% when all samples are counted. For IgM 7.6% and 9.4% respectively were found positive. There is a tendency to re-test patients with positive results.

The results here, however crude, reflect the lack of a gold standard for Lyme antibody testing. Thus it is difficult for the developers to adjust the cut-off of the assays. It will depend on the pre-selection of available evaluation samples (e.g. clinically defined erythema migrans, neuroborreliosis or pre-selection of samples positive in IB or ELISA assays used to estimate the analytic sensitivity. Furthermore, the diagnostic specificity will have a geographical component due to variations in endemicity and local clinical recommendations/preferences in the selection of patients for testing. The international guidelines propose the use of a two-tier strategy (1), however detailed recommendations/hints for the implementation are not given, as shown by the huge differences in reclassification rates from just a few (14%) to nearly all samples. This shows that it is not known how to adjust the analytic specificity and sensitivity of the first line test or the second line test. Thus the diagnosis of Lyme borreliosis still remains clinical with antibody testing as supportive indicator. Following this problem overview, it is important that both developers of diagnostic kits and clinical laboratories distinguish between the respective terms: on one hand analytical sensitivity and specificity, and on the other diagnostic sensitivity and specificity (8). In many kit evaluations and instructions for serological testing, it is not obvious that this is two different entities.

The differences in preferred methods chosen by the laboratories in Scandinavia were probably rational in consideration of the published evidence and the local conditions including reasonable costs and practical workflow in the laboratory. The use of IB-methods is labour-intensive and the gain is uncertain. As the final diagnosis remains clinical the important issue is to be aware of the rate of seropositivity local population. If for example 8% of blood donors are IgG positive then this figure may be used when advising on the clinical interpretation of the result especially in patients with atypical symptoms. Thus it is not so important if the specificity is for example 98% or 92%. The important matter for clinical decision-making is to be informed about the local specifity (9). As an example of the implementation of a local strategy, Åland island (Figure 1. lab 3) had chosen to implement an assay with a very highly sensitive assays to rule out both active infection with B. burgdorferi and immunity due to previous infection in the first tier assay, because of the very high (65% IgG) seroprevalence in parts of the population (10). As another example the specificity of the IDEIA – IgG was set at 98% in Danish blood donors as it was considered important not to have too many false positive when screening for Lyme borreliosis in patients with symptoms of longer duration, where antibody levels are expected to be very high above the cutoff in most cases(1) and pretest prevalence is low as Lyme arthritis and Acrodermatitis(11).

The possibility of false positive results, choice of cut-off, criteria for interpretation of IB and if a specific ELISA alone may be sufficient is also discussed in a recent European guideline (12).

For many years the only test for intrathecal antibody production on the Scandinavian market was the IDEIA(13-15). The capture ELISA principle is independent of the blood-barrier function. Recently other manufacturors have implemented protocols for testing spinal fluid, but published clinical studies are few and the need for separate assessment of blood barrier function requiring additional tests is under discussion.
The PCR results show that the most relevant material is joint fluid. A report on 23,777 consecutive routine specimens in the USA has found 6.4% PCR positive in synovial fluid and only rarely in blood and spinal fluid (16).

Conclusion

Rates of seropositivity in the routinely tested populations are different, this may be due to a combination of differences in assays, patient selection or geographic variation. The use of the two-tier principle is limited to 16 out of 43 laboratories and the selection criteria for two-tier analysis also vary. PCR is offered in 6 laboratories. The variation in rates of seroposivity between the different assays and the reclassification rates for two-tier-testing reflect the lack of more specific recommendations for borrelia-antibody testing. Further development of recommendations for the laboratory diagnosis of Lyme borreliosis could be considered.

This is a report of a survey and not a peer reviewed article and the design does not allow conclusions about the diagnostic accuracy of the different assays.

Transparancy declaration.

No funding was received concerning this manuscript.
Commercial companies marketing diagnostic products antendedthe Borrelia meeting in February 2009.Potential personal conflict of interests in relation to this study none to declare:
Tone Skarpaas, Dag Nyman and Ingvar Eliason
Ram Dessau: Collaboration with Oxoid/Thermofisher on evaluation of a Borrelia-ELISA assay. No personal income.

Literature

1. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of lyme borreliosis. Clin.Microbiol.Rev. 2005;18(3):484-509.

2. Brouqui P, Bacellar F, Baranton G, Birtles RJ, Bjoersdorff A, Blanco JR et al. Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. Clin.Microbiol.Infect 2004;10(12):1108-32.

3. Hauser U, Lehnert G, Lobentanzer R, Wilske B. Interpretation criteria for standardized Western blots for three European species of Borrelia burgdorferi sensu lato. J Clin.Microbiol. 1997;35(6):1433-44.

4. European Union Concerted Action on Lyme Borreliosis. http://meduni09.edis.at/eucalb/cms/index.php?lang=en . 2009.

5. Reiber H, Otto M, Trendelenburg C, Wormek A. Reporting cerebrospinal fluid data: knowledge base and interpretation software. Clin.Chem.Lab Med. 2001;39(4):324-32.

6. Tumani H, Nolker G, Reiber H. Relevance of cerebrospinal fluid variables for early diagnosis of neuroborreliosis. Neurology. 1995;45(9):1663-70.

7. Dessau RB. Comparison of C6 Immunetics with IDEIA-Oxoid, RecomWell-Mikrogen and Western Blot for Serologic Diagnosis of Lyme Disease. Poster SSAC, Copenhagen 2008.

8. Saah AJ, Hoover DR. "Sensitivity" and "specificity" reconsidered: the meaning of these terms in analytical and diagnostic settings. Ann.Intern.Med. 1997;126(1):91-4.

9. Skarpaas T, Ljostad U, Sobye M, Mygland A. Sensitivity and specificity of a commercial C6 peptide enzyme immuno assay in diagnosis of acute Lyme neuroborreliosis. Eur.J Clin.Microbiol.Infect Dis. 2007;.

10. Jansson C, Carlsson SA, Granlund H, Wahlberg P, Nyman D. Analysis of Borrelia burgdorferi IgG antibodies with a combination of IgG ELISA and VlsE C6 peptide ELISA. Clin Microbiol.Infect. 2005;11(2):147-50.

11. Dessau RB, Bangsborg JM, Ejlertsen T, Skarphedinsson S, Schonheyder HC. Utilization of serology for the diagnosis of suspected Lyme borreliosis in Denmark: Survey of patients seen in general practice. BMC.Infect.Dis. 2010;10(1):317.

12. Stanek G, Fingerle V, Hunfeld KP, Jaulhac B, Kaiser R, Krause A et al. Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe. Clin.Microbiol.Infect. 2010.

13. Hansen K, Lebech AM. Lyme neuroborreliosis: a new sensitive diagnostic assay for intrathecal synthesis of Borrelia burgdorferi--specific immunoglobulin G, A, and M. Ann Neurol. 1991;30(2):197-205.

14. Hansen K. Lyme neuroborreliosis: improvements of the laboratory diagnosis and a survey of epidemiological and clinical features in Denmark 1985-1990. Acta Neurol.Scand.Suppl 1994;151:1-44.

15. Hansen K, Lebech AM. The clinical and epidemiological profile of Lyme neuroborreliosis in Denmark 1985-1990. A prospective study of 187 patients with Borrelia burgdorferi specific intrathecal antibody production. Brain 1992;115 (Pt 2):399-423.

16. Babady NE, Sloan LM, Vetter EA, Patel R, Binnicker MJ. Percent positive rate of Lyme real-time polymerase chain reaction in blood, cerebrospinal fluid, synovial fluid, and tissue. Diagn.Microbiol.Infect.Dis. 2008;62(4):464-6.

Table 1. Number of laboratories participating in the survey for each country

Country	Number participating in the survey	Total number of laboratories performing borrelia serology
Danmark	15	15
Finland	2	6
Norge	11	15
Sverige	15	24
Total	43	57

Table 2. ELISA Assays used in Scandinavian laboratories on all samples received.

Name of assay	Number of laboratories	Short name
IDEIA IgG+IgM, Oxoid Cambridgeshire, UK.	16	IDEIA
Enzygnost Lyme link VlsE/IgG+Borrelia/IgM Siemens Health Care diagnostics, Marburg,Germany	8	Enzygnost
Liaison Borrelia IgG+IgM Saluggia, Italy	8	Liaison
Euroimmun Anti-Borrelia-plus-VlsE IgG+IgM	2	Euroimmun
C6 Immunetics, Boston,USA	2	C6
VIDAS Lyme IgM and IgG Biomerieux, Marcy l'Etoile, France	2	VIDAS
In-house B31 IgG+IgM	1	In house B31
In-house flagella IgG+IgM	1	Flagella
recomwell Borrelia IgG Mirogen, Floriansbogen, Germany and C6 immunetics	1	Mikrogen and C6
Virion/serion Elisa Borrelia burdorferi IgG/IgM, Würzburg, Germany	1	Virion
Premier™ Lyme (IgG/IgM), Meridian Bioscience, Cincinnati, USA	1	Premier
Total	43	

* Production statistics from 35 laboratories.

Table 3. Choice of ELISA with and without two-tier combinations.

	Second line assay	
Primary ELISA	**Immuno blot or ELISA**	**Number of Laboratories**
IDEIA		14
Enzygnost		5
Liaison		6
Liaison	Line Immunoblot Virotech, Rüsselheim, Germany	2
VIDAS	IDEIA	2
Enzygnost	recomBlot Mikrogen	2
IDEIA	Euroimmun Euroline WB, Lübeck Germany	1
In-house (Flagella)		1
Premier total	IDEIA	1
C6 immunetics		1
Enzygnost	recomBlot Mikrogen	1
C6 immunetics, Mikrogen IgG	recomBlot Mikrogen	1
IDEIA	Euroimmun Euroline WB	1
Euroimmun	Euroimmun Euroline WB	1
C6 immunetics	Line Immunoblot, Virotech	1
Euroimmun		1
Mikrogen	recomBlot Mikrogen	1
Virion/Serion		1
In-house B31	IDEIA and recomLine Mikrogen	1

Table 4. Assay's used for detection of intrathecal antibody production in the cerebrospinal fluid.

Assay	Number of laboratories
IDEIA	*33
Liaison	2
Enzygnost	2
In-house B31	1
In-house (Flagella)	1

*One laboratory uses Reiber-index for measurement of the blood brain barrier.

Table 5. Estimation of the average predictive values in % using healthy blood donors as the negative control group. TotPR are is the total rate of seropositivity from the routine production, FPR the rate of seropositivity in the local donor population, "PPV" is the estimated positive predictive value. Most of the statistical uncertainty in the PPV-estimate is from the relatively small number of blood donors tested. The 95% confidence interval for at binomial proportion e.g. 10/100 is about +/-5%.

Laboratory number (See Figure 1) and assay.	Number of blooddonor samples	IgG			IgM		
		TotPR	FPR	PPV	TotPR	FPR	PPV
2, C6	200	27.0	*16.0	40			
18, IDEIA	382	3.5	1.6	54	10.3	1.8	82
19, IDEIA	100	2.2	3.0	NC	11.8	3.0	74
22, IDEIA	91	6.9	1.1	84	9.1	1.1	87
30, Liaison	100	21.7	8.0	63	10.2	0.0	100
34,Liaison	100	25.0	7.0	72	8.8	3.0	65

*IgM or IgG, NC = not calculated

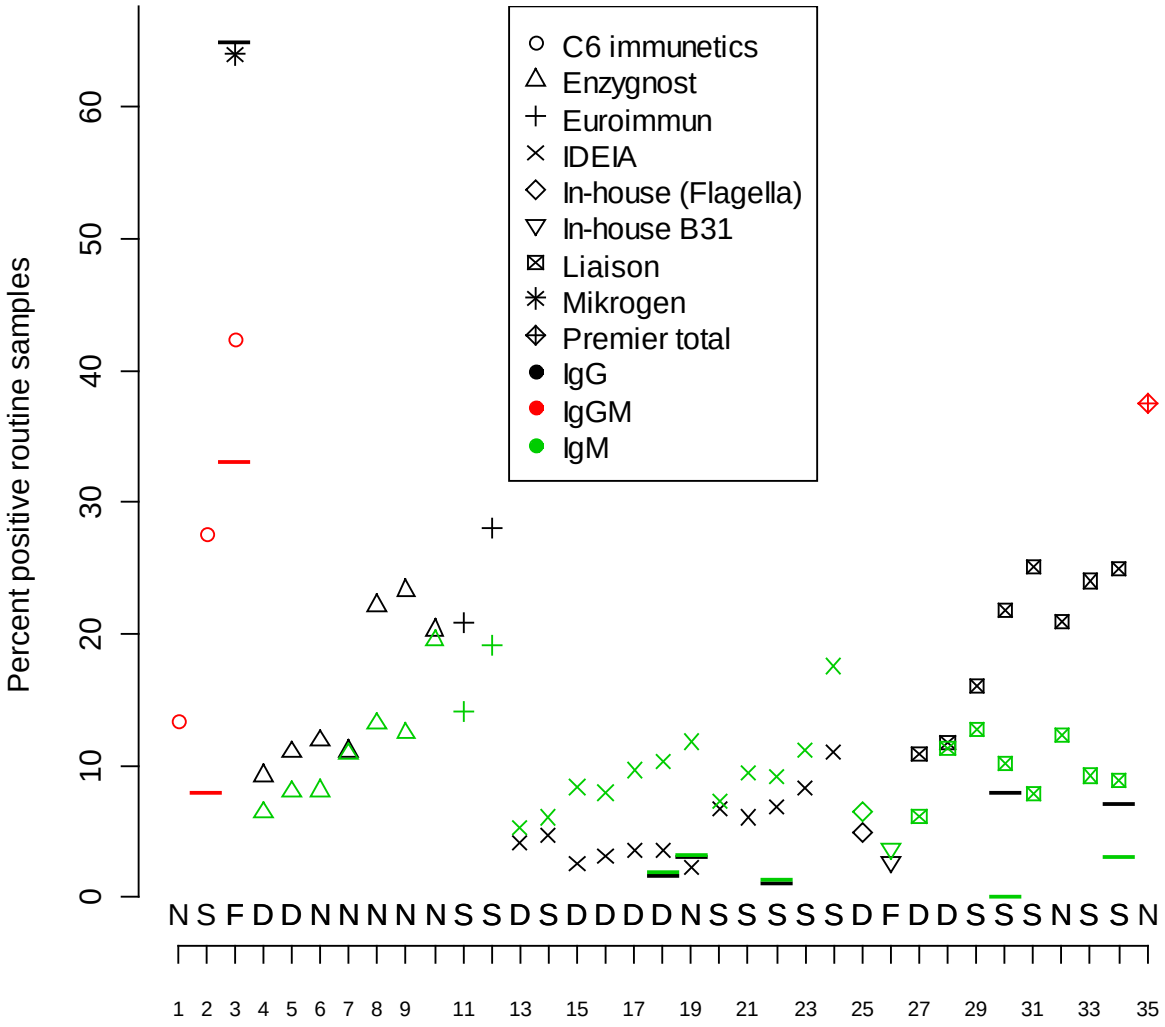

Figure 1. Assays used on all samples received and rate of seropositivity for IgG, IgM and the kits that do not distinguish the Ig-type (IgGM). Data from 35 laboratories with a minimum of 513 samples to a maximum of 14792 samples. The laboratories are sorted according to the name of the assay and the sum of the rate of seropositivity for IgG and IgM. From 7 laboratories rates of seropositivity in blood donors.are also shown (short horizontal lines). The Norwegian and Swedish laboratory at positition 19 and 23 have the same values for IgG and IgM. The color-coding is IgG(black), IgM(green) and kits which do not distinguish Ig-types (red). Denmark, Finland, Norway and Sweden are abbreviated by the first letter.

Participating laboratories (Contact person, Department, Town and Country):

Aandahl EH,Department of Microbiology, Innlandet Hospital Trust,Lillehammer,NO
Afset JE,Dept. of medical microbiology, St. Olavs Hospital,Trondheim,NO
Bangsborg JM,Herlev Hospital,Herlev,DK
Bohn C,Rigshospitalet,Copenhagen,DK
Bredberg A,Medical Microbiology, UMAS, Malmo, Sweden,Malmö,SE
Bäckman,Aleris Medilab,Täby,SE
Dzajic E,Region of Southern Denmark, Esbjerg Hospital,Esbjerg,DK
Ejlertsen TE,Department of Clinical
Microbiology, Aalborg Hospital, Aarhus University Hospital, ,Aalborg,DK
Emmert A,Mikrobiology Laboratory, Vestfold Hospital Trust,Tønsberg,NO
Ellermann-Eriksen E,Department of Clinical Microbiology, Aarhus University Hospital,Århus,DK
Forsum U,Clinical Microbiology Laboratory, Center for Laboratory Medicine, University Hospital Linköping,Linköping,SE
Hansen DS,Hillerød Hospital, Capital Region of Denmark,Hillerød,DK
Hjetlanad R,Department of Microbiology, Førde Central Hospital,Førde,NO
Holter E,Department of Microbiology, Oslo University Hospital Rikshospitalet,Oslo RH,NO
Hytönen J,University of Turku, Dept. of Medical Microbiology and Immunology,Turku,FI
Jansson C,Åland Central Hospital,Åland,FI
Jensen TG,Odense University Hospital,Odense,DK
Jonsson L,Laboratory of clinical microbiology, Södra Älvsborgs Hospital,Borås,SE
Jung K,Microbiology, Karolinska University Hospital,Solna,SE
Kajser B,Laboratory of clinical microbiology, Sahlgrenska University Hospital,Göteborg,SE
Kanestrøm A,Department of clinical microbiology, Østfold Hospital Trust,Østfold,NO
Kjaeldgaard P,Sønderborg Hospital, Region of Southern Denmark,Sønderbog,DK
Kjerstadius T,Department of Clinical Microbiology, Karlstad Hospital,,Karlstad,SE
Melby KK,Ullevål University Hospital ,Oslo,NO
Møller JK,Vejle Hospital, Region of Southern Denmark,Vejle,DK
Natås OB,Department of Medical Microbiology, Stavanger University Hospital ,Stavanger,NO
Nielsen IL,Department of biochemistry, Herning/Holstrebro,Holstebro,DK
Nils Grude,Unilabs Telelab,Skien,NO
Nilsson K,Clinical Microbiology, Uppsala University Hospital,Uppsala,SE
Noraas S,Sørlandet Hospital health enterprise ,Kristianssand,NO
Olcén P,Laboratory medicine, mikrobiology, Örebro University hospital,Örebro,SE
Pass MB,Department of clinical biochemistry, Roskilde Hospital, Region Zealand,Roskilde,DK
Ripa T,Department of Clinical Microbiology and Infection Control, Hospital of Halmstad,Halmstad,SE
Rova LP,Laboratory Medicine, Clinical Microbiology Karlskrona,Karlskrona,SE
Samdal HH,Department of microbiology, Buskerud hospital,
Vestre Viken Hospital Trust,Drammen,NO
Schiønning C,Hvidovre,Hvidovre,DK
Schön T,Department of Clinical Microbiology, Kalmar County Hospital,,Kalmar,SE
Sellin M,Clinical Bacteriology,Laboratory Medicine, Norrlands University Hospital,Umeå,SE
Tønning B,Department of clinical microbiology, Viborg Hospital,Viborg,DK
Villumsen S,Statens Serum Institut, Department of Bacteriology, Mycology, and Parasitology,Copenhagen,DK
Örvell C,Laboratory of Microbiology, Karolinska University Hospital,Huddinge,SE