Annual Report

1st April 2010-31st March 2011

Laboratory Service: Toxoplasma gondii

Provider: NHS Highland

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Director
Scottish Toxoplasma Reference Laboratory

Date: May 2011

1. Summary: Key Points

1. Clinical Pathology Accreditation (United Kingdom)
   The laboratory has full CPA Accreditation. There will be an interim inspection on 2nd June 2011.

2. Quality Assurance
   The high success rate in internal and external schemes demonstrates the quality of the service.

3. Activity
   The overall test activity was 36.5 % lower than predicted for the year reflecting the fall in samples from 1816 last year to 1450 this year. This was due to tightening the criteria for referral of samples.

4. Turnaround times
   100 % of test results were available within the required turnaround time of 7 working days.

5. Positive Results
   A breakdown of the serology and clinical results has been included to reflect the work of the laboratory.

6. Finance
   The financial outturn overspend was due to AFC reviews which were paid but not funded.

7. Reference Laboratories Contract
   The Reference Laboratory Review has been completed and our contract was renewed until 2013.

8. Cross charging Northern Ireland
   Agreement has been reached and charging will be implemented from 1st April 2011.

9. CE Marking
   Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in-vitro* Diagnostic Medical Devices is being reviewed . There is concern that 'in house' tests will no longer be exempt from CE marking.

2 Activity data

2.1 Contracted activity

Test	Contracted number	Actual number
ELISA IgG	1278	985
ELISA - BAM	1278	985
DYE TEST	2662	1666
ISAGA IgM	315	8
IgA	343	20
IgE	10	
IgG AVIDITY	115	107
WESTERN BLOTTING	4	8
TOXOREAGENT LATEX	78	39
*AXSYM– IgM	208	166
		3984
PCR ISOLATION	70	54
		54
TOTAL	6361	4038

*performed to support the IgG avidity test

Previous years: 5977 tests in 2001-2, 7226 tests in 2002-3, 6933 tests in 2003-4, 7505 tests in 2004-5, 6900 tests in 2005-6, 6628 tests in 2006-7, 6317 tests in 2007-8 , 6495 in 2008-9 and 5741 in 2009-10.

The overall test activity of 4038 was 36.5% lower than predicted for the year and 29.7% lower than last year. This reflects a fall of 366 in the number of samples from 1816 to 1450. This fall is largely due to the fall in referrals from Greater Glasgow from 603 last year to 247 this year. This reflects the change in referral policy adopted by the Regional Virus Laboratory in July 2010 (Section 3.4.5).

The number of ISAGA tests has continued to fall from the contracted number of 668 to 94 last year and 28 this year. Use of the ISAGA has been suspended. The kits are expensive and the test is used only in very special circumstances. The Western blot has replaced the ISAGA for the investigation of congenital toxoplasmosis and the fall in sample numbers means the kits cannot be used before their expiry date. The requirement for the ISAGA will be monitored.

2.2 Activity by Health Board of referral

Health Board	No. of Samples (%) Current Year	No. of Samples (%) Previous Year
AYRSHIRE & ARRAN	4 (0.3)	14 (0.8)
BORDERS	0 (0)	1 (0.1)
DUMFRIES & GALLOWAY	62(4.3)	64(3.5)
FIFE	20(1.4)	16(0.9)
GRAMPIAN	50(3.4)	43(2.4)
GREATER GLASGOW	247(17.0)	603(33.2)
LANARKSHIRE	113 (7.8)	114(6.3)
LOTHIAN	227 (15.7)	252 (13.9)
TAYSIDE	35(2.4)	97(5.3)
HIGHLAND & WESTERN ISLES	197(13.6)	273(15.0)
NORTHERN IRELAND	495 (34.1)*	339 (18.7)
SWANSEA REFERENCE UNIT	0 (0.0)	0 (0.0)
	1450(100)	1816(100)

*This represents test activity of 14.1% as they screen.

Activity in previous years: 1318 samples in 2001-2, 1645 samples in 2002-3, 1842 samples in 2003-4, 2004 samples in 2004-5, 1920 samples in 2005-6, 1970 samples in 2006-7 , 1948 in 2007-8, and 1946 in 2008-2009. There is now a return to 2001-2 sample levels.

Overall here has been a 20 % fall in the number of samples referred. This is largely due to the 59% fall (603 to 247)in the number of samples from Greater Glasgow Health Board. This was the result of the Regional Virus Laboratory, Gartnavel Hospital, Glasgow tightening their referral criteria.

There has been a 46% increase in number of samples from Northern Ireland but activity has been maintained below the agreed level of 15%: 9% in 2001-2, 9.5% in 2002-3, 8.6% in 2003-4, 9.4% in 2004-5, 10.5% in 2005-6, 8.8% in 2006-7, 12% in 2007-8 ,9.2% in 2008-9, 7.1 % in 2009-10 and 14.1% in 2010-11. The rise in samples in 2010-11 probably reflects the reorganization and centralization of laboratory services which has taken place in Northern Ireland and may not be sustained. It has been agreed that from 1st April 2011 Northern Ireland will be charged for toxoplasma testing.

Positive Results

2.3.1 Serology results

		Toxoplasma antibody		
Patient group	No	negative	Equivocal	Positive
Glands	157	78 (49.7%)		77(49.0%)
CNS	17	11(64.7%)	2(11.8%)	4(23.5%)
BMT	42	18 (42.9%)	8 (19.0%)	16 (38.1%)
Donor/Recipient	140	65 (46.4%)	16 (11.4%)	59 (42.1%)
HIV/ high risk/ immunocompromised	144	85 (59.0%)	11 (7.6%)	48 (33.3%)
Ocular	141	55 (39.0%)	18 (12.8%)	68 (48.2%)
Pregnancy	241	129 (53.5%)		112 (46.5%)
Congenital	101	84 (83.2%)		17 (16.8%)
Other clinical	188	87 (46.3%)	6 (3.2 %)	95 (50.5%)
No details	258	112 (43.4 %)	7 (2.7%)	139 (53.9%)
Total	1429	724 (50.7 %)	68 (4.8%)	635 (44.4%)

*21 insufficient to test

2.3.2 Clinical results

a) Bone Marrow Transplants**:** 18 negative results make toxoplasma reactivation unlikely, 16 positive results makes reactivation a major consideration and 8 equivocal results need retesting.

b) Donor/ recipient**:** in 124 cases mismatches can be avoided and in 16 equivocal cases there needs to be further testing.

c) HIV/ High Risk/ Immunocompromised**:** in 85 cases reactivation is not a problem, in 48 cases reactivation has to be seriously considered and in 11 equivocal cases further testing is required.

d) Ocular**:** in 55 cases the negative serology is incompatible with congenital ocular toxoplasmosis, in 68 cases results support a clinical diagnosis of congenital toxoplasmosis and in 18 cases there needs to be repeat serology.

e) Pregnancy**:** in 129 cases there is no evidence of past infection and mothers must be given advice to avoid infection and in 112 cases there is evidence of past infection and the mother is immune or further tests are required to exclude current infection.

f) Congenital**:** in 84 cases there is no evidence of congenital infection and 17 cases there is supporting evidence.

g) Current infection**:** 28 cases; 23 in Scotland (Dumfries 1, Fife 4 , Grampian 3, Inverclyde 2, Lothian 11, Tayside 1, Highland 1) and 5 in Northern Ireland
19 female and 9 male : 15 glands, 1 HIV, 2 immunocompromised, 3 ocular, 2 pregnant women , 1 tiredness, 1 granulomatous disease, 1 jaundice, 2 no details (NB. None from Greater Glasgow)
Raised dye test: 13 cases; 10 in Scotland (Fife 1, Grampian 1, Lothian 5, Tayside 1, Glasgow 2) and 3 in Northern Ireland.; 9 female and 4 male ;4 glands, 3 HIV, 2 ocular , 1 kidney donor , 1 pregnant, 1 lymphocytosis, 1 no details

3 Quality Assurance Report

3.1. Turn around times by test category

Test	No	No (%) with turnaround time in days		
		≤ 2 days	≤ 7 days	> 7 days
1. ELISA IgG	985	705 (71.6)	280 (28.4)	0
ELISA BAM	985	684 (69.4)	301 (30.6)	0
DYE Test	1666	1379 (82.8)	287 (17.2)	0
TOTAL	**3636**	**2768 (76.1)**	**868(23.9)**	**0**
2. IgG Avidity	107	13 (12.2)	94 (87.8)	0
ISAGA IgM	8	2 (25.0)	6 (75.0)	0
IgA	20	2 (10.0)	18 (90.0)	0
IgE	0	0	0	0
Mast Latex	39	21 (53.8)	18 (46.2)	0
Axsym-IgM	166	45 (27.1)	121 (72.9)	
TOTAL	**340**	**83 (24.4)**	**257 (75.6)**	**0**
3. Western blotting	8	0 (0)	8 (100)	0
PCR	54	29(53.7)	25 (46.3)	0
* Isolation	0	0	0	0
TOTAL	**4038**	**2880 (71.3)**	**1158 (28.7)**	**0**

1. First line serological tests
2. Secondary confirmatory tests
3. Tests being monitored

* Not applicable minimum time 4-6 weeks

For first line tests 76.1% of results were available within 2 days. For the dye test 82.8 % of results were available within 2 days. Overall, 100% of results were available within the contract level of 7 working days.

3.2. Internal audit (Appendix A)

3.2.1 Horizontal audit: 2 audits performed: sick leave.

3.2.2. Vertical audit: 1 audit was performed : Toxoplasma dye test

3.2.3. Examination audit: 3 audits performed: preparation for and storage of samples in liquid nitrogen, AxSym standard set up procedure, AxSym internal quality control monitoring..

3.2.4 Internal Quality Assurance

10 samples: recycled in10 distributions of 1 sample.
35 tests: ELISA IgG (10), BAM ELISA (10), Dye test (10), AxSym IgM (4), IgG avidity (1)

There were no differences in test selection, results or interpretation.

3.2.5 Clinical Effectiveness
These were joint audits with the Virology department.

3.2.5.1 An audit to introduce an internal control for sample extraction and PCR. The Primerdesign DNA extraction control kit was evaluated. The system works well and can be carried out in the same tube as the target amplification making it cost-effective. This is an important improvement and fulfills CPA requirements .

3.2.5.2 An audit to review use of reagents in PCR. Reagents were routinely aliquoted in amounts sufficient for 8 tubes. With the internal control there is no need for seeded controls and 4 tubes are sufficient to test 1-2 samples. Reducing the reagent aliquots reduces wastage and cost.

3.2.5.3 An audit to replace diluters with pipettes for the toxoplasma dye test. Preliminary results showed that direct replacement was not possible and the test required recalibration. This was more difficult as the established WHO standard for calibration was discontinued and a new standard was not available. Extensive parallel testing and exchange of sera with the Swansea Toxoplasma Unit confirmed pipettes can be used for the dye test.

3.2.5.4 An audit to assess the requirement for replacement of the shaking waterbath for preparation of biotin labelled toxoplasma antigen found a static waterbath could be used. The shaking waterbath will not be replaced.

3.2.5.5 An audit to assess the use of microtitre strips for the Toxoplasma ELISA tests. There was excellent agreement between strips and plates for the ELISA G but the BAM ELISA gave false positives in strips. Furtheremore despite wasted wells in plates testing in strips would be significantly more expensive.

3.2.5.6 An audit of recovery of toxoplasma tachyzoites from liquid nitrogen. Maintaining passage of cultures is costly and time consuming. Passage will stop after 21 days if tachyzoite harvests > $1x10^6$ /ml are not being produced and after 60 days if dye test quality harvests are not being produced. This shorter recovery time is much more appropriate as back-up for the culture system.

3.2.5.7 An audit to evaluate the toxoplasma questionnaires. The return rate was 31% for the ocular questionnaire and 51% for the current infection questionnaire. The ocular questionnaire was complex and this probably contributed to the poor return rate. A new single page questionnaire was brought into use in April 2011.

3.3 Accreditation Schemes (Appendix B)

Name of Scheme	Current status	Date/type of last visit	Date /type of next visit
Clinical Pathology Accreditation (UK) Ltd	Full Accreditation CPA Reference: 0492	1st / 2nd July 2009 Full Inspection	2nd June 2011 Interim visit

There were no Reference Laboratory non compliances or observations.
The letter and certificate of CPA Accreditation was received in November 2009.

3.4 External Audit Schemes (Appendix C)

3.4.1 UK NEQAS Toxoplasma IgG Serology
18 samples, 3 distributions of 6 samples Pre and post distribution testing
126 tests: ELISA IgG (36), Dye test (36), Mast Toxoreagent (36), AxSym IgG (18)
Success rate: 100%

3.4.2 UK NEQAS Toxoplasma IgM Serology
8 samples, 2 distributions of 4 samples. Pre and post distribution testing
32 tests: BAM ELISA IgM (16), AxSym IgM (16)
Success rate: 100%

3.4.3 National Toxoplasma Reference Unit Serology
20 samples, 2 distributions of 10 samples
104 tests: ELISA IgG (20), Dye test (20), Mast Toxoreagent (20), BAM ELISA IgM (20), AxSym IgM (15), IgG avidity (9).
There were 2 discrepancies: IgM tests.
Success rate: 98.1%

3.4.4 National Toxoplasma Reference Unit PCR

8 samples, 2 distributions of 4 samples (1000, 100, 10, 0 toxoplasma tachyzoites)
Success rate: 100%

3.4.5 Audit with Users

At the Toxoplasma Reference Laboratory Meeting in February 2010 the laboratory was asked to explore tightening the criteria for referral of samples. This was discussed by the Virology Consultants at the Scottish Diagnostic Virology Meeting in Inverness in May 2010. Evaluation studies were undertaken with the Gartnavel Laboratory , Glasgow and Foresterhill ,Aberdeen to compare their screening assays with the dye test. The results demonstrated that the tests gave positive results for samples with dye test $\geq$ 15 iu/ml but gave false negative results for 60-80% of samples with dye test results 2-8 iu/ml. Demonstration of low level antibody is particularly important in immunocompromised patients. Therefore in addition to referring all ocular and possible acute samples our recommendation was to refer all negative and equivocal samples from BMT, transplant and HIV patients for dye test to establish a baseline and subsequently test 'in-house' and refer only on the basis of clinical symptoms or change in result.

3.5 Incidents and Complaints

There have been no incidents or complaints.

3.6 Equality and diversity impact assessments

Paul Nairn Service Planning Manager and Jean Chatterton Principal Clinical Scientist completed an Equality and Diversity Impact Assessment on 12th March 2010. The EQIA checklist was completed satisfactorily and identified no requirement for change. There have been no changes in staff or procedures.

4. New developments

4.1 We have introduced an internal control for the extraction and amplification steps of the PCR. This improves quality control and fulfils the requirement for CPA.

4.2 There have been changes in sample referral as a result of the audit (Section 3.4.5). The Regional Virus Laboratory , Gartnavel Hospital, Glasgow now limits referral to ocular patients and possible acute infection; BMT and HIV patients are now only tested 'in-house'. These changes have reduced the number of samples they refer by 59% from 603 last year to 247 this year. Referrals from Aberdeen increased by 16.3% from 43 last year to 50 this year.

4.3 From 1st April 2011 Northern Ireland will be charged for Toxoplasma testing.

5. Future developments

5.1 There may be a need to CE mark 'in- house' tests. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in-vitro* Diagnostic Medical Devices is being reviewed. Currently 'in-house' tests as used in the Reference Laboratory are exempt from CE marking. If this exemption is not maintained laboratories would not be able to access Reference Laboratory tests until they were CE marked. This would compromise the diagnosis of toxoplasma infection in Scotland. The laboratory has made a written response to HPS and National Services Division and we await the outcome.

5.2 Preparation of antigen for the in-house IgM ELISA from cell culture tachyzoites. A method to produce the required numbers of tachyzoites is being developed.

5.3 The standard for the Toxoplasma NEQAS PCR scheme has now been produced. This will be distributed to an international panel of 40 laboratories for evaluation.

5.4 Assessment of immunoblotting to improve the serological diagnosis of ocular toxoplasmosis.

6 Teaching

6.1 Internal Staff Training

Type of training	Details	Numbers attending
Practical	Refresher training for BMS staff rotating between Virology and Toxoplasma	2
Discussion/ audit	Monthly Clinical Effectiveness Meeting	12
Problems/ planning	Monthly staff meetings	6
Presentation/ discussion	(i) Clinical Effectiveness Meeting April 2010 Paper discussion: 'The United Kingdom National External Quality Assessment Scheme for Parasitology: Toxoplasma Serology Subschemes.'	12
	(ii) Journal Club May 2010 Presentation/discussion: 'Neutralization assay to resolve discrepancies between positive results in new highly sensitive anti- Toxoplasma gondii IgG assays and negative results in reference tests.'	12
	(iii)Microbiology Monthly Meeting June 2010 Presentation: 'Risk factors for toxoplasma infection'	30
	(iv) MLA Training June 2010 Presentation/Discussion: 'Toxoplasmosis'	6

6.2 Individual Placements

Visitor	Grade	Speciality	Base Place of Work	Duration of placements
Gillian Slack	Trainee Clinical Scientist	Microbiology	Microbiology Ninewells Hospital Dundee	19th-23rdt April 2010.

6.3 External Training

Type of training	Topic	Numbers attending
Presentation Scottish Diagnostic Virology Group Meeting May 2010	Risk factors for toxoplasma infection	98

6.4 Publications

(i) Chatterton JMW, McDonagh S, Ho-Yen DO. Toxoplasma tachyzoites from cell culture are more appropriate in some situations. J Clin Path 2010 ;63:438-440.

(ii) Manser MM, Chatterton JMW, Guy E, Francis J, Holliman R, Ho- Yen DO, Johnson J, Chiodini PL. The United Kingdom National External Quality Assessment Scheme for Parasitology: Toxoplasma Serology Subschemes. J Clin Path 2010; 63:1112-1115.

(iii) Chatterton JMW, McDonagh S, Spence N, Ho-Yen D O. Changes in Toxoplasma Diagnosis. J Med Micro (submitted for publication)

7 **Research**

7.1 Investigation of effect of long term maintenance of Toxoplasma gondii tachyzoites in cell culture.
7.2 Use of cell culture- derived tachyzoites in BAM-IgM ELISA and ISAGA tests.
7.3 Development of cell culture techniques for isolation of toxoplasma.
7.4 Investigation of adaptation of Toxoplasma gondii tachyzoites to cell culture.

8 Staffing

8.1 There have been no staff problems or changes.

10 Summary and conclusions

The Scottish Toxoplasma Reference Laboratory test activity was lower than last year. This decrease was due to the fall in sample numbers as a result of tightening the referral criteria and the reduction of ISAGA tests. Success in internal and external quality assurance schemes confirms the quality of the service. Salary costs have increased as a result of Agenda for Change and staff are grateful to NSD for funding regradings Efficiency savings have been made and this will remain a priority for the laboratory.

11. Appendices

Appendix A Internal Audit Exception Reports/ Risk Register

1 Error log

1.1 Two reports were sent to the wrong location. Corrected reports were issued , staff were informed and reminded to check demographics.

1.2 One sample was booked in for the wrong procedure which resulted in a short delay in testing. Staff were informed and reminded to check test requests.

2 Risk register

2.1 An updated risk log is attached
There have been no changes.

Appendix C External QA Exception Reports

National Toxoplasma Reference Unit Serology

1. There were two IgM exceptions:

1.1 The Inverness result for one sample was IgM positive by BAM ELISA and negative by AxSym ; the Swansea laboratory result was borderline IgM. Testing a second aliquot confirmed the BAM ELISA results and the AxSym IgM was a low positive

1.2 The Inverness result for one sample was IgM negative by BAM ELISA and positive by AxSym ; the Swansea laboratory result was negative. Testing a second aliquot confirmed the Inverness results.

Reference Laboratory Services Risk Log: May 2011

Laboratory	Risk	Required action to mitigate risk	Responsible Lead	Deadline	RAG Status	Comment
Scottish Toxoplasma Reference Laboratory	**Staff illness** The laboratory has a small number of staff and has already experienced periods of extended sick- leave	Integration within the Virology/Parasitology Department provides the staff support when required to maintain the service	Dr DO Ho-Yen	In place	?	
	Cell culture failure The laboratory relies on a continuous culture system to provide the toxoplasma tachyzoites for in- house tests. In particular for the dye test	The laboratory has considerable stocks of cultures and cells in liquid nitrogen storage. There is a rolling recovery programme to ensure the quality of the stored material. If this recovery system failed cultures could be obtained from the Toxoplasma Reference unit in Swansea to maintain the system	Dr D O Ho- Yen	In place	?	
	Information management system failure Used for results processing and reporting	The Medipath system is used throughout Microbiology and is supported by the IT department	Dr D O Ho-Yen			