

From: Collette, Crystal <Crystal.Collette@dfo-mpo.gc.ca>
Sent: Friday, September 9, 2011 5:17 AM
To: Hawley, Laura <Laura.Hawley@dfo-mpo.gc.ca>
Cc: Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Gagne, Nellie <Nellie.Gagne@dfo-mpo.gc.ca>; Roy, Mélanie V <Melanie.Roy@dfo-mpo.gc.ca>
Subject: RE: ISAV Proficiency Panel
Attach: 2011P-ISA-01 2011-08-30.mxp

Hi Laura,

After review of your ISA proficiency panel, we have determined that you have passed. Congratulations!
However, we did observe that the threshold was not set in the linear part of the curve. We would suggest that the threshold be placed a bit above the background within the 3rd lower part of the linear curve, as well as above any irregularities in the curve.

Please see attached Mxpro data file for reference.

<<...>>

We also have a few questions regarding proficiency panel Ct value determination. We would like to make sure that the criteria used to determine the accepted value range are similar or identical to yours.

How many assays are done on the proficiency panel samples to determine the accepted range of values?
Do the assay include one single extraction, and multiple PCR testing on that same panel, or are multiple extractions done in one batch (or multiple batches)??

Also, what would be the criteria used to determine the accepted range of values? (i.e. standard deviation, etc)

Thank you,
Have a great weekend,

Crystal & Mélanie

Crystal Collette

Technician Molecular Biology Unit/Technicienne Unité Biologie Moléculaire
Fisheries and Oceans Canada (Gulf Region)/Pêches et Océans Canada (Région Gulf)
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From: Hawley, Laura
Sent: 2011--August-30 6:30 PM
To: Collette, Crystal
Cc: Garver, Kyle
Subject: RE: ISAV Proficiency Panel

Hi Crystal,
Attached is the result form and the MxPro file for ISA proficiency panel 2011P-ISA-01.

Cheers,
Laura

<< File: LH-PCR-269 2011P-ISA-01 MS-088 2011-08-30.mxp >> << File: PBS--Laura Hawley 2011-08-23 CC.doc >>

-----Original Message-----

From: Collette, Crystal
Sent: August 25, 2011 3:28 AM
To: Hawley, Laura
Cc: Gagne, Nellie
Subject: RE: ISAV Proficiency Panel

Hi Laura,

Thank you for the email.
I am very happy that the samples arrived to you in good condition.
Have a great end of week,

CC

Crystal Collette

Technician Molecular Biology Unit/Technicienne Unité Biologie Moléculaire
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From: Hawley, Laura
Sent: 2011--August-24 4:10 PM
To: Collette, Crystal
Cc: Gagne, Nellie
Subject: RE: ISAV Proficiency Panel

Hi Crystal,

Just got the ISAV panels, all in good shape. Hopefully I can have a panel processed and results back to you by the end of next week. I've attached the form with the shipping section filled out and will complete the remainder when I have results

Thanks,
Laura

<< File: PBS--Laura Hawley 2011-08-23 CC.doc >>

-----Original Message-----

From: Collette, Crystal
Sent: August 23, 2011 10:52 AM
To: Hawley, Laura
Cc: Gagne, Nellie
Subject: RE: ISAV Proficiency Panel

Hi Laura,

I have sent the ISAV panels. Attached you will find a Proficiency testing form, I ask that you fill out the information and email me back the form. I have also sent a paper copy with the shipment.

If you have any questions please do not hesitate to ask. (You should be receiving the sample tomorrow, as I sent them via express post) Please let me know when you receive the samples, and in what condition they have arrived.

<< File: PBS--Laura Hawley 2011-08-23 CC.doc >>

Thanks,
Crystal

Crystal Collette

Technician Molecular Biology Unit/Technicienne Unité Biologie Moléculaire
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From: Hawley, Laura
Sent: 2011-August-23 11:36 AM
To: Collette, Crystal

Subject: RE: ISAV Proficiency Panel

Thanks Crystal
The panels can be sent to the following address

Laura Hawley
Pacific Biological Station (T302)
3190 Hammond Bay Rd.
NANAIMO, BC V9T 6N7
Ph 250-756-7131

Cheers,
Laura

-----Original Message-----

From: Collette, Crystal
Sent: August 23, 2011 4:57 AM
To: Hawley, Laura
Cc: Gagne, Nellie
Subject: RE: ISAV Proficiency Panel

Hi Laura,

I will be sending you two ISAV proficiency panels by the end of the day , you should receive them tommrow,may I have your contact information (complete mailing adresse and phone number)?

Thanks,
Crystal

Crystal Collette

Technician Molecular Biology Unit/Technicienne Unité Biologie Moléculaire
Fisheries and Oceans Canada (Gulf Region)/Pêches et Oceans Canada (Région Gulf)
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From: Gagne, Nellie
Sent: 2011-August-19 8:28 AM
To: Collette, Crystal

Subject: RE: ISAV Proficiency Panel

From: Hawley, Laura

Sent: 17 août 2011 18:46

To: Gagne, Nellie

Subject: ISAV Proficiency Panel

Hi Nellie,

Would it be possible to get two ISAV proficiency panels (next week or so)? In reading the ISAV protocol I noticed a statement that different RNA extraction protocols can be used if proven proficient. My plan is to do one proficiency panel following GFC extraction protocol and another following our extraction protocol. Hopefully both will yield equivalent results, which would give me the option to do either extraction method when ISAV testing is of interest.

I have been looking at the protocol and SOPs that are in Q-pulse, please also email if there are any changes to the protocols/SOPs that may not have made it to Q-pulse yet.

Thank you,
Laura

MxPro - Mx3000P

File DFO-6025 3201-01.mxp

Multiplex Quantitative PCR Systems

Quantitative PCR - Consolidated Report

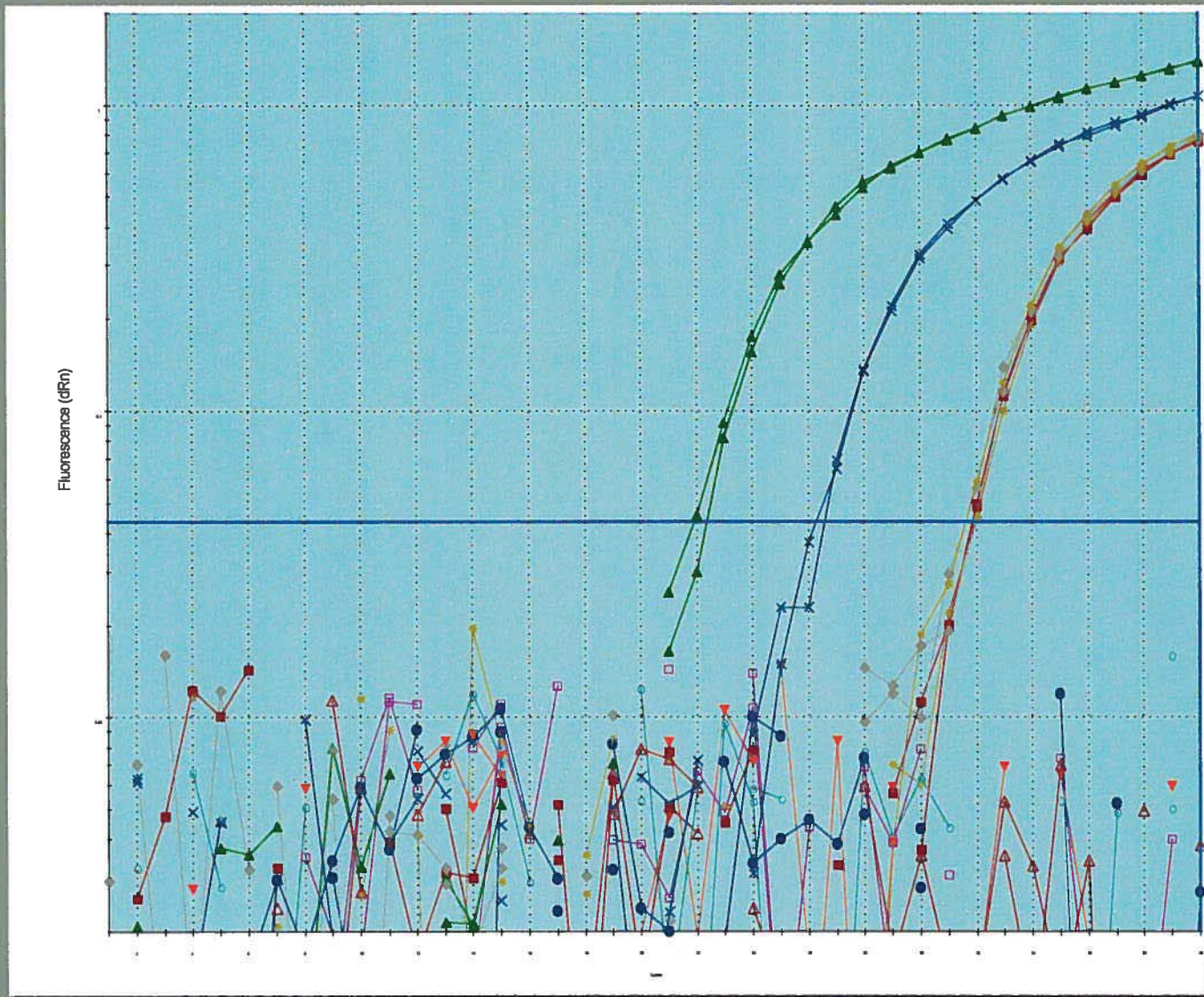
C:\Documents and Settings\hawley\Local Settings\Temporary Internet Files\OLKB1\2011P-ISA-01 2011-08-30.mxp

Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x4

Run date: August 30, 2011

Replicates: Treated individually

Amplification Plots



MxPro - Mx3000P

File: *DF0-6025³²⁰¹302-01.mxp*

Multiplex Quantitative PCR Systems
 Quantitative PCR - Consolidated Report

C:\Documents and Settings\hawley\Local Settings\Temporary Internet Files\OLKB1\2011P-ISA-01 2011-08-30.mxp

Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x4

Run date: August 30, 2011

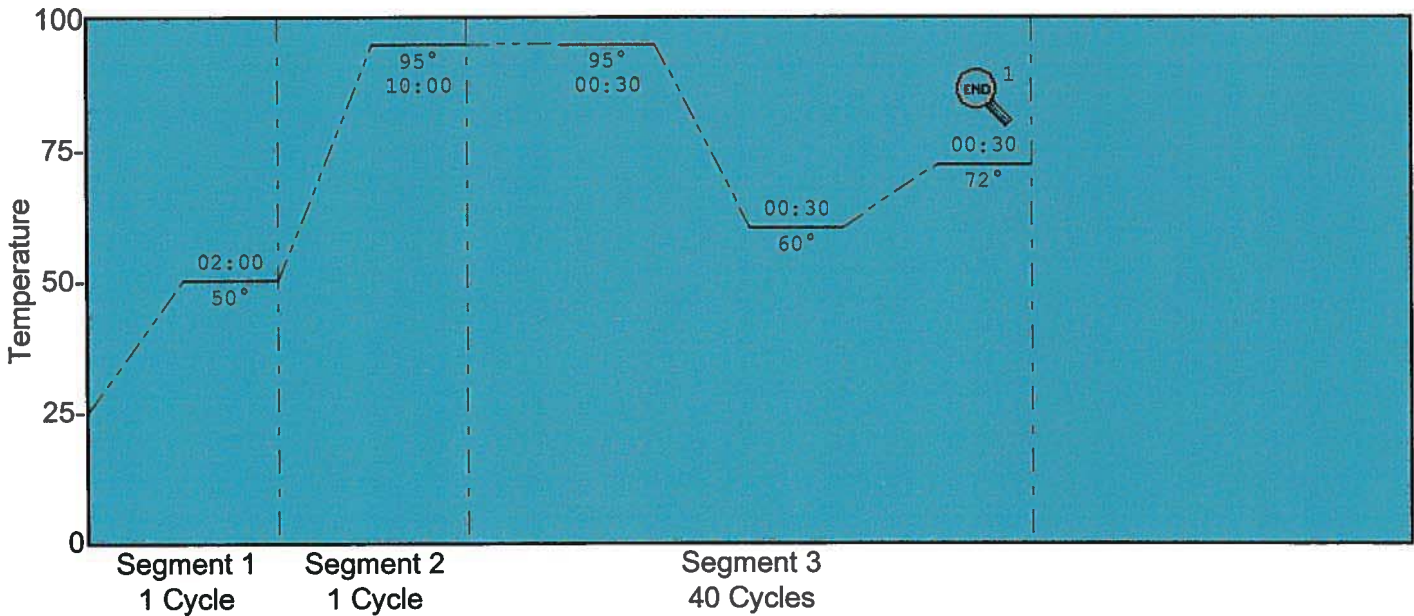
Replicates: Treated individually

Plate Setup

	1	2	3	4	5	6	7	8	9	10	11	12
	Unknown	Unknown	FAM Pos	FAM Pos								
A	REF 1 FAM	REF 1 FAM	REF 9 FAM	REF 9 FAM								
	Unknown	Unknown	NTC	NTC								
B	REF 2 FAM	REF 2 FAM	REF 10 FAM	REF 10 FAM								
	Unknown	Unknown										
C	REF 3 FAM	REF 3 FAM										
	Unknown	Unknown										
D	REF 4 FAM	REF 4 FAM										
	Unknown	Unknown										
E	REF 5 FAM	REF 5 FAM										
	Unknown	Unknown										
F	REF 6 FAM	REF 6 FAM										
	NTC	NTC										
G	REF 7 FAM	REF 7 FAM										
	NTC	NTC										
H	REF 8 FAM	REF 8 FAM										

Thermal Profile

(Estimated Run Time: 01:43:38)



MxPro - Mx3000P

Multiplex Quantitative PCR Systems
Quantitative PCR - Text report

C:\Documents and Settings\hawley\Local Settings\Temporary Internet Files\OLKB1\2011P-ISA-01 2011-08-30.mxp

Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x4

Run date: August 30, 2011

File: DFO-6025²⁰¹3002-01.mxp

Thermal Profile Summary

Segment	Cycles	Plateau	Temp. (degrees)	Temp. Inc. (deg/sec)	Duration (min:sec)	Time Inc. (min:sec)	Collect
1	1	Plateau 1	50.0	0.0	02:00	00:00	<none>
2	1	Plateau 1	95.0	0.0	10:00	00:00	<none>
3	40	Plateau 1	95.0	0.0	00:30	00:00	<none>
3	40	Plateau 2	60.0	0.0	00:30	00:00	<none>
3	40	Plateau 3	72.0	0.0	00:30	00:00	1 Endpoints

Replicates: Treated individually

* Fluorescence term used: dRn

Text Report

Well	Well Name	Well Type	Replicate	Threshold*	Ct*
A1	2011P-ISA-01 #1	Unknown	1	Reference	Reference
A1	2011P-ISA-01 #1	Unknown	1	0.0437	No Ct
A2	2011P-ISA-01 #1	Unknown	1	Reference	Reference
A2	2011P-ISA-01 #1	Unknown	1	0.0437	No Ct
B1	2011P-ISA-01 #2	Unknown	2	Reference	Reference
B1	2011P-ISA-01 #2	Unknown	2	0.0437	31.85
B2	2011P-ISA-01 #2	Unknown	2	Reference	Reference
B2	2011P-ISA-01 #2	Unknown	2	0.0437	31.88
C1	2011P-ISA-01 #3	Unknown	3	Reference	Reference
C1	2011P-ISA-01 #3	Unknown	3	0.0437	22.38
C2	2011P-ISA-01 #3	Unknown	3	Reference	Reference
C2	2011P-ISA-01 #3	Unknown	3	0.0437	21.93
D1	2011P-ISA-01 #4	Unknown	4	Reference	Reference
D1	2011P-ISA-01 #4	Unknown	4	0.0437	31.61
D2	2011P-ISA-01 #4	Unknown	4	Reference	Reference
D2	2011P-ISA-01 #4	Unknown	4	0.0437	31.98
E1	2011P-ISA-01 #5	Unknown	5	Reference	Reference
E1	2011P-ISA-01 #5	Unknown	5	0.0437	31.61
E2	2011P-ISA-01 #5	Unknown	5	Reference	Reference
E2	2011P-ISA-01 #5	Unknown	5	0.0437	31.95
F1	2011P-ISA-01 #6	Unknown	6	Reference	Reference
F1	2011P-ISA-01 #6	Unknown	6	0.0437	No Ct
F2	2011P-ISA-01 #6	Unknown	6	Reference	Reference
F2	2011P-ISA-01 #6	Unknown	6	0.0437	No Ct
G1	extraction neg	NTC	7	Reference	Reference
G1	extraction neg	NTC	7	0.0437	No Ct
G2	extraction neg	NTC	7	Reference	Reference
G2	extraction neg	NTC	7	0.0437	No Ct
H1	cDNA neg	NTC	8	Reference	Reference
H1	cDNA neg	NTC	8	0.0437	No Ct

MxPro - Mx3000P

Multiplex Quantitative PCR Systems

Quantitative PCR - Text report

C:\Documents and Settings\hawley\Local Settings\Temporary Internet Files\OLKB1\2011P-ISA-01 2011-08-30.mxp

Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x4

Run date: August 30, 2011

File: DFD-6025302-01.mxp²⁰¹

Well	Well Name	Well Type	Replicate	Threshold*	Ct*
H2	cDNA neg	NTC	8	Reference	Reference
H2	cDNA neg	NTC	8	0.0437	No Ct
A3	qPCR pos	FAM Positive Control	9	Reference	Reference
A3	qPCR pos	FAM Positive Control	9	0.0437	26.59
A4	qPCR pos	FAM Positive Control	9	Reference	Reference
A4	qPCR pos	FAM Positive Control	9	0.0437	26.29
B3	qPCR neg	NTC	10	Reference	Reference
B3	qPCR neg	NTC	10	0.0437	No Ct
B4	qPCR neg	NTC	10	Reference	Reference
B4	qPCR neg	NTC	10	0.0437	No Ct