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Seed is Life

Detection of *Xanthomonas hortorum* pv. *carotae* in Carrot Seed

Comparative test report, August 2021

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CONTENTS

Detection of *Xanthomonas hortorum* pv. *carotae* in Carrot Seed

1. INTRODUCTION

Xanthomonas hortorum pv. *carotae* (Xhc) is the causal agent of bacterial blight of carrot (*Daucus carota* subsp. *sativus*). It is a common problem wherever carrots are grown, and it can reduce carrot yields and seed quality. Xhc is commonly seedborne, and contaminated seeds are an important inoculum source for development of bacterial blight in the field (Umesh *et al.*, 1998). The relationship between the overall level of carrot seed contaminated with Xhc, and the incidence and severity of carrot bacterial blight was determined under field conditions in Davis, California (Umesh *et al.*, 1998). The seed transmission threshold for the establishment of Xhc populations on leaves and for the development of carrot bacterial blight was determined to be 10^4 to 10^5 CFU/gram of seed, under the environmental conditions that occurred in the area during the study (low rainfall and relative humidity).

An important control measure for bacterial blight of carrot is the use of sufficiently healthy carrot seed. ISTA method 7-020, a dilution plating method using semi-selective media for the detection of Xhc, was published in 2006. It was derived from previously performed inter-laboratory comparative tests and validation studies carried out by ISHI-Veg in 2003 (Asma, 2005).

A TaqMan based qPCR assay was developed and validated by ISHI-Veg to replace the conventional gel-based PCR in the current ISTA Rule 7-020 (Oosterhof, 2019). The inter-laboratory comparative test focused on the qPCR assay, which replaced the conventional gel-based assay and was published in version 2 of the ISHI-Veg protocol for the Detection of Xhc on carrot seed (ISF, 2019). The qPCR assay was composed of a triplex of three primer sets, two Xhc-specific TaqMan primer sets that bind to two different independent loci, developed by Barnhoorn (2014) and Temple *et al.* (2013). The third primer set that is Gram stain specific and developed by Wu *et al.* (2008) (the 'Wu' primers) serves as an internal amplification control (IAC) in the qPCR assay.

There is an increasing use of Seed Extract qPCR (SE-qPCR) methods in seed health testing, as SE-qPCR is sensitive, specific, and fast. However, SE-qPCR is an indirect method in that it detects the presence of nucleic acids (DNA) that are specific to the target pathogen in a seed extract without isolating the actual bacterium. It does not discriminate between viable and dead cells. Nevertheless, indirect methods may be used as a pre-screen prior to the application of a direct test, such as the existing dilution plating assay using semi-selective media, giving seed companies an additional tool for risk assessment (see ISF's position on indirect seed health tests).

An SE-qPCR method for detecting Xhc based on two Xhc-specific TaqMan primer sets has been jointly developed by several vegetable seed companies. It uses the bacterium *Acidovorax cattleyae* (Acat) as an Internal Amplification Control (IAC) and Positive Extraction Control (PEC).

An ISHI-Veg inter-laboratory comparative test using the SE-qPCR method was organized in 2018 with ten ISHI-Veg member laboratories. The original aim of the comparative test was to validate SE-qPCR as a pre-screen assay for the detection of Xhc in untreated carrot seeds. For comparison purposes, testing in tandem was conducted with the existing dilution plating assay together with the recently validated ISHI-Veg qPCR assay. The pathogenicity assay in contrast, was not included in the comparative test.

Conclusions of the inter-laboratory comparative test and the validation studies conducted within ISHI-Veg member laboratories revealed that the SE-qPCR was not fit for purpose as a pre-screen assay (Internal report 2021, in prep.). However, results obtained with the existing dilution plating assay together with the recently validated ISHI-Veg qPCR assay were used to confirm their combined repeatability and reproducibility within the ISHI-Veg method for 'Detection of *Xanthomonas hortorum pv. carotae* on Carrot Seed', version 2.

2. OBJECTIVES

The aim of this report is to assess the repeatability and reproducibility of the ISHI-Veg method for 'Detection of *Xanthomonas hortorum* pv. *carotae* on Carrot Seed', version 2, based on the data generated during the SE-qPCR inter-laboratory comparative test performed in 2018. The diagnostic sensitivity and specificity were also evaluated. The assay was validated according to the ISHI-Veg guidelines for the Validation of Seed Health Tests (Version 3, November 2020). The other performance criteria, i.e., analytical specificity and sensitivity, selectivity and diagnostic performance were evaluated and validated previously (Asma, 2005 and Oosterhof, 2019).

3. INTER-LABORATORY COMPARATIVE TEST

An ISHI-Veg inter-laboratory comparative test was organized in 2018 by Vilmorin-Mikado (France) and Bejo Zaden (The Netherlands) in which ten laboratories participated (Table 1). The test plan followed for this comparative test, including seed samples, controls (positive and negative control descriptions), statistical analyses and methods, can be found in Annex A.

Table 1. Participating laboratories in the Xhc inter-laboratory comparative test in 2018.

Laboratory	Contact person
Bejo Zaden	M. Asma*
Vilmorin	A. Lê Van*
Bayer	B. Geraats
CSP Labs	P. Sudarshana
Eurofins	R. Chitrampalam
GEVES	T. Baldwin
Naktuinbouw	M. Bruinsma
Rijk Zwaan	J. Oosterhof
Sakata Seeds JP	Y. Hosobuchi
Sakata Seeds US	C. Hogan

*Test organisers

All participants received a set of coded seed samples between March and June 2018. The set was comprised of 15 samples each with 10,000 untreated carrot seeds with one of three levels of infection (none or healthy, medium and highly contaminated). In addition to 15 seed samples, each laboratory received a series of controls (i.e. IAC, PPC, NPC, PAC, & NTC) as described in Annex A. Each laboratory followed the ISHI-Veg method for 'Detection of *Xanthomonas hortorum* pv. *carotae* on Carrot Seed', version 2, for the dilution plating assay and for the newly validated qPCR confirmation assay for the identification of suspect colonies. Each laboratory used its own qPCR equipment and reagents.

3.1. Homogeneity test

Methods

For each infected seed lot, representing two levels of infection (medium and high), 10 sub-samples were tested per infection level for the homogeneity test, using the dilution plating and the qPCR assays as described in Annex A. For the healthy seed lot, 5 sub-samples were tested for the homogeneity test (instead of 8 to 10) due to a late need to replace the healthy seed lot. However, 8 samples were still analysed in the stability test (see section 3.2).

Statistical analysis

Using the ISTA tool, Seedcalc8 (ISTA, 2007), the infection rate was estimated for each non-homogenous seed lot based on the estimated infection rate and a probability of 95%. The expected number of positive subsamples for each non-homogenous seed lot was calculated with another tool from the ISTA toolbox for seed health, "Probability of k positive samples out of n" (https://www.seedtest.org/en/seed-health-tool-box-_content---1--3450.html).

Results

All sub-samples from the healthy seed lot were negative. Eight out of 10 and 10 out of 10 sub-samples from the medium and highly contaminated seed lots were positive, respectively (Table 2). The mean number of CFU/g of seeds was calculated from positive sub-samples for each level of infection. It was $9x10^2$ CFU/g and $7.3x10^4$ CFU/g for the medium and highly infected seed lots, respectively. Using Seedcalc8 the infection rate was estimated for the medium level of infection seed lot B. The computed % in sample at 95% of confidence interval (CI) gave an estimate contamination rate of infected seed in seed lot B of 0.02%. The expected number of positives among the nine subsamples of seed lot B shared with the participants in the comparative test (see section 3.4) was 6 to 9 positive batches at 95% confidence level (Table 3).

Table 2. Results of the homogeneity test per seed lot using the dilution plating method. Results are presented as the number of positive subsamples over the total number of tested subsamples.

Seed Lot	Xhc Infection level	Obtained results
A	Healthy	0/5
B	Medium	8/10
C	High	10/10

Table 3. Probability to find k positive samples out of n (n=9 subsamples of seed lot B shared with the participants in the comparative test, see section 3.4) given the estimated infection rate of 0.02% and 10,000 seeds per sample. The probability was calculated using the "Probability of k positive samples out of n" tools from the ISTA ToolBox for Seed Health.

k	Probability (%) of *k* positive out of *n*	*k*	Probability (%) of *k* positive out of *n*
0	0.0000	5	2.0416
1	0.0001	6	8.6980
2	0.0022	7	23.8221
3	0.0333	8	38.0590
4	0.3195	9	27.0241

Conclusion

The healthy and highly contaminated seed lots were homogeneous. For the medium infected seed lot, the expected number of positive seed samples for each laboratory in the comparative test was calculated to be between 6 to 9, taking into account a probability of 95% and the estimated infection rate obtained from the homogeneity test for the medium infected seed lot.

3.2. Stability test

Methods

Fourteen subsamples were tested for the stability test, 8 for the healthy seed lot, and 3 each for the medium and highly contaminated seed lots using the dilution plating and the qPCR assays as described in Annex A.

Statistical analysis

The observed number of positive subsamples per Xhc infection level was compared to the expected number of positive subsamples as calculated based on the homogeneity test results (see section 3.1, Table 3).

Results

All healthy subsamples were negative. All three highly infected subsamples were positive. Two out of three subsamples from the medium contaminated seed lot were positive (Table 4). The probability to detect two positive subsamples out of three was above 5% (Table 5).

Table 4. Results of the stability test per seed lot using the dilution plating method. Results are presented as the number of positive subsamples over the total number of tested subsamples. Expected results for the stability test are calculated according to the homogeneity test results.

Seed Lot	Xhc Infection level	Obtained result	Expected results
A	Healthy	0/8	0/8
B	Medium	2/3	2 to 3/3
C	High	3/3	3/3

Table 5. Probability to find k positive samples out of n (n=3) given the estimated infection rate of 0.02% and 10,000 seeds per sample. The probability was calculated using the "Probability of k positive samples out of n" tools from the ISTA ToolBox for Seed Health.

k	Probability (%) of *k* positive out of *n*
0	0.2477
1	4.7493
2	30.3507
3	64.6523

Conclusion

The infection rate was stable, and the selected seed batches met the stability requirements for this comparative test.

3.3. Diagnostic performance

Definition of the diagnostic performance ISHI-Veg guidelines: *An evaluation of the ability of the method to discriminate between positive and negative seed lots*

As the dilution plating and qPCR identification assays are preceding the pathogenicity assay, the diagnostic sensitivity (no false negatives) is more important than the diagnostic specificity (no false positives), as false positive will be detected by the pathogenicity assay.

Although there is no fixed rule, values above 95% are considered acceptable for analytical sensitivity, analytical specificity and accuracy (ISTA, 2018).

The diagnostic performance requirements will therefore be met when diagnostic sensitivity, and accuracy will be above 95% and the diagnostic specificity is above 90%. A lower requirement was set for the specificity as the dilution plating and qPCR identification assays are preceding the pathogenicity assay and false positive are acceptable.

Experimental approach

Samples were processed by each laboratory following the protocol given in Annex A. Data from the comparative test were analysed according to the Standard NF EN ISO 16140 (AFNOR, 2003). Sensitivity, specificity, and accuracy of the method, (diagnostic performance of the assay), were calculated according to the following mathematical formulas:

	Expected result +	**Expected result -**
Obtained result +	positive agreement (PA) +/+	positive deviation (PD) -/+
Obtained result -	negative deviation (ND) +/-	negative agreement (NA) -/-

Diagnostic Sensitivity = ΣPA / (ΣPA+ΣND) x 100

Diagnostic Specificity = ΣNA / (ΣNA+ΣPD) x 100

Accuracy = (ΣNA+ΣPA) / (ΣPA+ΣNA+ΣPD+ΣND) x 100

Results

The data obtained by each laboratory are provided in Annex D. All seed samples from the highly contaminated seed lot were detected by all laboratories. For the medium contaminated seed lot all laboratories found the expected number of positive seed samples. For the healthy samples, out of ten laboratories, two found one out of three samples as positive (Table 6).

Laboratory A found on the healthy seed sample (sample 10), 8 suspect colonies in 3 dilutions on the MKM media (Annex C). After subculturing, four colonies remained suspects, and after qPCR one colony was positive (Cq values of 20.94 and 20.86 for the MVS and Temple specific primers, respectively; and Cq value of 16.85 for the Wu IAC primers).

Laboratory G found on the healthy seed sample (sample 1), 1 suspect colony in 1 dilution on the MKM media (Annex C). After subculturing, the colony remained suspect and after qPCR it was positive. However, the Cq values were high for the specific primer sets (Cq values of 31.22 and 29.44 for the MVS and Temple specific primers, respectively; and Cq value of 17.35 for the Wu IAC primers). For this laboratory, among the PPC, the maximum Cq values obtained using the MVS and Temple primers were 20.58 and 16.73, respectively. The maximum Cq value for the Wu primers for the positive process control was 17.28.

Table 6. Expected and obtained Xhc dilution plating results per laboratory for the healthy, medium and highly Xhc infected carrot seed lots. Expected number of positive samples was based on the homogeneity test. Deviations from the number of expected detects are indicated in red.

Laboratory code	Seed lot A healthy	Seed lot B medium	Seed lot C high
expected	*0/3*	*6 to 9/9*	*3/3*
A	1/3	9/9	3/3
B	0/3	6/9	3/3
C	0/3	7/9	3/3
D	0/3	8/9	3/3
E	0/3	8/9	3/3
F	0/3	8/9	3/3
G	1/3	8/9	3/3
H	0/3	9/9	3/3
I	0/3	9/9	3/3
J	0/3	9/9	3/3
Sum of positives	*2*	*81*	*30*

The diagnostic sensitivity was calculated according to the expected results concluded from the homogeneity test (i.e., if a laboratory found six out of nine positive samples in the medium level of contamination then it was considered to be in accordance with expectation, and was not counted as a negative deviation). Based on this, the diagnostic sensitivity was 100%; the diagnostic specificity was 93%, and the accuracy of the method was 99% (Table 7).

Table 7. Number of agreement and deviations.

	Expected result +	Expected result -
Obtained result +	positive agreement (PA) = 111	positive deviation (PD) = 2
Obtained result -	negative deviation (ND) = 0	negative agreement (NA) = 28

Sensitivity = ΣPA / (ΣPA+ΣND) x 100 = 111/(111+0) x 100 = 100%

Specificity = ΣNA / (ΣNA+ΣPD) x 100 = 28/(28+2) x 100 = 93.3%

Accuracy = (ΣNA+ΣPA) / (ΣPA+ΣNA+ΣPD+ΣND) x 100 = (111+28)/(111+28+2+0) x 100 = 98.6%

Discussion

Out of the ten participating laboratories, all but two laboratories had negative results with the healthy samples. These two laboratories found one positive seed sample among the three healthy samples tested. For both laboratories only one of the suspected colonies was positive after qPCR identification. For laboratory G, the Cq values for the specific primers were high compared to the positive process control, and a cross contamination was suspected. However, it is not possible to distinguish a very low contamination level from false positive due to cross contaminations. The homogeneity analysis for the healthy seed lot was performed on 5 sub-samples instead of 10, due to replacement of the healthy seed lot at the last moment, and a very low contamination level in the healthy seed lot cannot be excluded based on the homogeneity analysis carried out. However, during the stability test, 8 samples were analysed, and all were negative. The two positive samples found in the healthy seed lot led to a diagnostic specificity at 93% which is still above the requirement (90%).

Conclusion

With a sensitivity of 100%, specificity of 93% and an accuracy of 99% the requirements were met.

3.4. Repeatability (accordance) and Reproducibility (concordance)

Definition repeatability ISHI-Veg guidelines: *Degree of similarity in results of replicates of the same seed lots when the method is performed with minimal variations in a single laboratory.*

Definition reproducibility ISHI-Veg guidelines: *Degree of similarity in results when the method is performed across laboratories with replicates of the same subsamples.*

The requirements for repeatability and reproducibility will be met when the accordance and concordance of the test results obtained by the different laboratories on the tested samples are above the accepted values of 90%.

Experimental approach

Samples were processed by each laboratory following the protocol given in Annex A. Accordance (repeatability of qualitative data) and concordance (reproducibility of qualitative data) were evaluated using the method developed by Langton *et al.* (2002). Results were analysed separately for each level of infection using the ISTA online tool based on Langton's method (https://www.seedtest.org/en/tool-box-_content---1--1410.html). The default parameters were used (i.e., representative bootstrap method, 5,000 bootstraps and 95% confidence limits).

Samples from the same seed lot were considered as replicates. The number of replicates was 3 for the healthy and the highly contaminated seed lots and 9 for the medium contaminated seed lot. The heterogeneity of the medium infected seed lot was considered, with the number of expected positive samples at this infection level, to be between 6 to 9 (section 3.1).

A medium level of infection was used, instead of an infection level just above the limit of detection. This medium infection level was already below the threshold needed to observe disease in field (Umesh *et al.*, 1998). In addition, with a lower infection level the homogeneity would also be lower and the number of samples needed to be tested would be higher. Therefore, the medium level of infection was chosen as a compromise between the level of infection and the homogeneity of a seed sample.

Results

For the highly contaminated (3 samples) and the medium contaminated (9 samples) samples the accordance and the concordance were 100% with a Concordance Odds Ratio (COR) value of 1 (Table 8). For the healthy samples (3 samples) the accordance (87%) and the concordance (87%) were below requirements (Table 8). The COR value was 0.96.

Table 8. Accordance, concordance, and COR ratio for each level of infection and their lower and upper limits calculated using a representative bootstrap method with 5,000 iterations and a 95% confidence interval. Values below requirements are indicated in red (ideally, the COR ratio is 1).

Level of infection	Accordance		Concordance		COR ratio	
	Estimate (%)	Lower-upper limits	Estimate (%)	Lower-upper limits	Estimate	Lower-upper limits
Healthy	87	67 - 100	87	72 - 100	0.96	0.79 - 1
Medium	98	93 - 100	100	100 - 100	1	1 - 1
High	100	100 - 100	100	100 - 100	1	1 - 1

Discussion

Requirements for the healthy level was not met due to one false positive (out of three samples) obtained in two laboratories out of 10 (A and G). These results were discussed above in the diagnostic performance (section 3.3). For laboratory G, the Cq values were high which would rather indicate a cross contamination in the PCR or DNA extraction. Laboratory G was an unexperienced laboratory which did not use in a routine basis the dilution plating assay for the detection of Xhc. Deviation from the expected results could be explained by these factors. Furthermore, cross contaminations during sample preparation of the comparative test or carry over in sample processing are also potential sources. We cannot exclude that these false positives were a trace of infection in the heathy batch not detected during the homogeneity and the stability tests. However, these issues are connected to process control rather than the method performance.

Conclusion

For the healthy level, the set requirement was not met due to the diagnostic specificity issue discussed above. Presence of one false positive out of three samples obtained in two out of the ten participating laboratories lowered the accordance and concordance performance to below the threshold. However, accordance and concordance requirements were met for the medium and high level of contamination and therefore method performance is still acceptable.

4. CONCLUSION

The dilution plating assay combined with the new qPCR assay for colony confirmation, described in the ISHI-Veg method for 'Detection of *Xanthomonas hortorum* pv. *carotae* on Carrot Seed', version 2, was found to be fit for purpose for the detection of Xhc. The Diagnostic Performance met the requirements and also the accordance and concordance met the requirements when considering the medium and high level of contamination (Table 9). Considering the healthy sample, the set requirements for accordance and concordance were not met due to the unexpected detection of suspected target Xhc in one (out of three) healthy sample in two of the ten participating laboratories. We cannot conclude from the data if this is due to a trace infection in the healthy batch, a cross contamination in the respective laboratory, carry over during sample preparation of the comparative test, or a false positive qPCR result detecting non-Xhc strains. However, all positive samples after the qPCR identification assay should be confirmed by the pathogenicity assay which was not included in this comparative test. As false positive results are less critical than false negative results in assays that are followed up by a direct assay, we conclude that the method is fit for purpose.

Table 9. Performance criteria of the dilution plating and the qPCR identification assays per infection level category.

Infection level	Diagnostic Sensitivity	Diagnostic Specificity	Accordance	Concordance
Healthy	N.A.	93%	87%	87%
Medium	100%	N.A.	100%	100%
High			100%	100%

N.A.: not applicable

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6. ANNEXES

Annex A. Inter-laboratory comparative test plan

ISHI-Veg inter-laboratory comparative test plan for the detection of *Xanthomonas hortorum* pv. *carotae* on carrot seeds using a Seed Extract qPCR Assay as a pre-screen

Crop: Carrot (*Daucus carota* subsp. *sativus*)
Pathogen: *Xanthomonas hortorum* pv. *carotae* (Xhc)
Date: February 2018

1. Organization

1.1 Test Organisers

Margreet Asma Bejo Zaden B.V. Trambaan 1c 1749 CZ Warmenhuizen - The Netherlands	Rodolphe Germain Vilmorin SA Route du Manoir 49250 La Ménitré - France

1.2 Participating laboratories and contact persons

Laboratory	Contact person
Bejo Zaden	M. Asma*
Vilmorin	R. Germain*
Bactochem**	N. Bikson
Bayer	B. Geraats
CSP Labs	P. Sudarshana
Eurofins	P. Chitrampalam
GEVES	T. Baldwin
Naktuinbouw	M. Bruinsma
Rijk Zwaan	J. Oosterhof
Sakata Seeds JP	Y. Hosobuchi
Sakata Seeds US	C. Hogan

*Test organisers; ** Could not provide results for the comparative test due to human resources issues.

Criteria required

Laboratories experienced with seed health testing and use of molecular techniques for detection of bacteria, in particular *Xanthomonas hortorum* pv. *carotae* (Xhc).

1.3 Time line

Time	Action	Person
June 2017	Draft test plan to ITG	Test organisers
August-December 2017	Finalizing test plan	Test organisers
January 2018	Final test plan to ISTA	Test organisers
November-February 2018	Selection seed lots	Test organisers
March-June 2018	Testing samples comparative test	Participants
July-December	Data analysis and writing report	Test organisers + ITG
ISHI meeting 2018	Presentation results	Test organisers
ISHI meeting 2019	ISTA validation	Test organisers

2. Introduction and objective of this test plan

2.1 Background

Xanthomonas hortorum pv*. carotae* (Xhc) is the causal agent of bacterial blight of carrots (*Daucus carota* subsp*. sativus*). It is a common problem wherever carrots are grown, and it can reduce carrot yields and seed quality. Xhc is commonly seedborne and contaminated seeds are an important inoculum source for development of bacterial blight in the field (Umesh *et al.*, 1998). The relationship between the level of carrot seed infected with Xhc and the incidence and severity of carrot bacterial blight was determined under field conditions in Davis, California (Umesh *et al.*, 1998). The threshold of seed infection for the establishment of *X. hortorum* pv*. carotae* populations on leaves and for the development of carrot bacterial blight was 10^4 to 10^5 CFU/gram of seed, under the environmental conditions that occurred in the area during the study (low rainfall and relative humidity).

2.2 Detection in Seed

An important means of control of the disease is the use of sufficiently healthy carrot seed. ISTA method 7-020, a dilution plating method using semi-selective media for the detection of Xhc, was published in 2006. It was derived from previously performed comparative tests and validation studies carried out by ISHI-Veg in 2003 (Asma, 2005).

There is an increasing use of Seed Extract qPCR (SE-qPCR) methods in seed health testing, as they are sensitive, specific and fast. SE-qPCR is, however, an indirect method in that it detects the presence of nucleic acids (DNA) that are specific to the target pathogen in a seed extract without isolating the actual bacterium. It does not discriminate between viable and dead cells.

Indirect methods may, nevertheless, be used as a pre-screen prior to the application of a direct test, such as the existing dilution plating assay using semi-selected media, giving seed companies an additional tool for risk assessment (see ISF's position on indirect seed health tests, http://www.worldseed.org/resources/position-papers/#specifc-technical-subjects).

An SE-qPCR method for detecting Xhc based on two Xhc-specific TaqMan primer sets that bind at two different independent loci, developed by Barnhoorn (2014) and Temple *et al.* (2013), has been jointly developed by several vegetable seed companies. It uses the bacterium *Acidovorax cattleyae* (Acat) as an Internal Amplification Control (IAC) and Positive Extraction Control (PEC). The Acat is added as a spike to the seed wash just after incubation but before filtration to monitor the extraction of DNA and amplification in qPCR.

2.3 Aim and objective of the inter-laboratory comparative test

The aim of this inter-laboratory comparative test is to validate the SE-qPCR as a pre-screen in the detection of Xhc in untreated carrot seeds, and demonstrate that a sample that gives a negative SE-qPCR result also gives a negative result with dilution plating, i.e. the Limit of Detection (LOD) of SE-qPCR is the same as or lower than that of the dilution plating assay. Any positive result with a pre-screen will be classified as a "suspect" result and will be investigated further using the confirmatory test, i.e. the dilution plating assay, for either a positive (infected) or negative result (healthy).

Laboratories are required to strictly follow the method described below except where it is explicitly stated that optimization or lab specific settings are allowed/required. In case a participating lab wishes to use an alternative step or method (e.g. DNA-extraction kits), it must be done in parallel to the described method but **NOT** as a replacement.

3. Material and methods

3.1 Seed samples and controls

Each participating laboratory will analyse a set of 15 samples of 10,000 untreated carrot seeds each. The set will be composed of healthy seed samples and naturally infected seed samples from seed lots with medium and high infection levels of Xhc. The samples of 10,000 seeds will be prepared using the thousand-seed-weight (TSW). The 15 samples will be **coded** and their identity will only be known to the test organiser(s).

Each participating lab will also receive an inactivated (boiled) Acat culture that will be used as an Internal Amplification Control (IAC) to validate a negative PCR result and also serve as a Positive Extraction Control (PEC). The initial concentration of Acat bacteria provided to each participating laboratory will be approximately 10^7-10^8 CFU/mL.

A Positive Process Control (PPC) and a Negative Process Control (NPC) will also be included in the test package, as well as a Positive Amplification Control (PAC) for both Xhc and Acat, and a Non-Template Control (NTC).

Controls provided by the Test organiser

IAC: Internal Amplification Control	Inactivated (boiled) suspension of Acat for early spiking
PPC: Positive Process Control	Xhc positive seed sample
NPC: Negative Process Control	Xhc negative seed sample
PAC-Xhc: Positive Amplification Control	1. Boiled Xhc bacterial suspension 2. DNA extract from Xhc isolate 3. DNA extract from Xhc positive seed sample (only for SE-qPCR)
PAC-Acat: Positive Amplification Control	DNA extract from *Acidovorax cattleyae* (only for SE-qPCR)
NTC: Non Template Control	Nucleic acid-free water

The 15 samples of carrot seeds, PPC and NPC must be stored between 4-15 °C until processing since temperature may influence the stability of the pathogen in the seed samples. The controls IAC, PAC and NTC must be stored at -20 °C immediately upon arrival.

3.2 Statistical analysis

A homogeneity and stability test will be performed by the Test Organiser before and after the inter-laboratory comparative test on seed samples of each seed lot, the PPC and the NPC following the ISTA *Guidelines for organizing and analysing results of Proficiency tests (PT) and inter laboratory tests for validation of methods (CT)* (ISTA Seed Health Committee, 2013).

Data will be checked for outliers using box-plots and tested for normality.

The qualitative analysis according to the method developed by Langton *et al.* (2002) (see https://www.seedtest.org/en/tool-box-_content---1--1410.html) will be done per infection level: final results of a sample being a positive, i.e. a suspect till viability and pathogenicity is determined, or negative Xhc detection, using accordance and concordance as described by Langton *et al.* (2002) will be compared for the SE-qPCR and dilution plating assays.

Furthermore, a quantitative analysis using ANOVA will also be performed on the qPCR data to show the potential of the SE-qPCR method to discriminate between heavily, medium infected and healthy seed samples.

The controls will be checked at quantification cycle (Cq)-level with box-whisker plots to detect possible deviants over laboratories. In case of deviants for the PC or NC, Cq-values will be subjected to ANOVA to investigate if these are significantly different at the lab-level. In case of differences between laboratories, further checks will be carried out to determine if this can be linked to factors other than the laboratory itself, such as PCR machine, PCR mix, etc. (given that enough laboratories have used the different methods to allow for a good comparison).

3.3 Methods

Each laboratory will perform SE-qPCR and dilution plating on each seed sample.

The results should be recorded in Appendix 1 and returned to the Test Organiser. The PCR reaction mixtures used should also be specified in Appendix 1.

The protocols and materials needed for SE-qPCR and dilution plating are described below. For dilution plating ISTA Rule 7-020 must be followed. However, for the confirmation of suspected colonies a triplex qPCR assay should be used (see section 3.3.6.) instead of gel-based PCR described in sections 8.2-8.7 of the ISTA Rule 7-020. The pathogenicity step (7.1-7.5 of the ISTA Rule 7-020) is not included in this inter-laboratory comparative test.

In order to make it possible to perform an additional gel-based PCR as described in ISTA Rule 7-020 at a later point in time, the cell suspension of the suspected colonies must be kept at -20 °C.

3.3.1. Materials needed

- For dilution plating: see also ISTA Rule 7-020
- Known strain of *Xanthomonas hortorum* pv. *carotae* (Xhc)
- Sterile Seed Extraction Buffer (sSEB)

Ingredient	Quantity for 1 L
Sodium chloride (NaCl)	8.5 g
Tween 20	0.2 mL
Demineralised water	1,000 mL

- Downflow cabinet with UV light (preferred) or Laminar airflow cabinet as alternative
- Microliter pipettes (e.g. Gilson, Finn) with sterile filter pipette tips (1 µL – 5 mL)
- Glassware (e.g. flasks, conical flask etc.)
- Sterile syringe and micropore membrane syringe filter (5 µm)
- Laboratory equipment (e.g. shaker, pH meter, magnetic stirrer etc.)
- Oligonucleotides for Xhc (Barnhoorn, 2014, Temple *et al.*, 2013), Acat (Koenraadt *et al.*, 2014) and Gram Strain specific (Wu *et al.*, 2008).

❑ **Note**: The participants can make their own choice for the dye and quencher of the probes (marked with asterix *)

- Oligonucleotides MVS-Xhc: Barnhoorn (2014)

Oligonucleotide	**Sequence 5'-3'**
MVSXhc-3F	CCA.AAg.CAg.TCg.CAA.ACT.TgA
MVSXhc-3R	AAT.TgC.ggA.TTC.CCA.ACA.AA
MVSXhc-3P*	VIC-Tgg.CCC.TAA.gCT.TCA.A-NFQ-MGB

- Oligonucleotides Temple-Xhc : Temple *et al.* (2013)

Oligonucleotide	Sequence 5'-3'
Xhc-q2F	gCA.TgA.AGG.CAA.TAC.AgC.g
Xhc-q2R	CgA.TCC.AgC.TGA.TgT.TCT.CCg.AA
Xhc-q2P*	6FAM-TCA.AgC.TCA.gAC.gAA.ACC.ggC.gTC-BHQ1

- Oligonucleotides Acat: Koenraadt *et al.* (2014)

Oligonucleotide	Sequence 5'-3'
Acat-2F	TgT.AgC.gAT.CCT.TCA.CAA.g
Acat-2R	TgT.CgA.TAg.ATg.CTC.ACA.AT
Acat-1P*	HEX-CTT.gCT.CTg.CTT.CTC.TAT.CAC.g-BHQ1

- Oligonucleotides Gram Strain Specific: Wu *et al.* (2008)

Oligonucleotide	Sequence 5'-3'
Wu-F	CAA.CgC.gAA.gAA.CCT.TAC.C
Wu-R	ACg.TCA.TCC.CCA.CCT.TCC
Wu-P1*	TxRd-ACg.ACA.ACC.ATg.CAC.CAC.CTg-BHQ2
Wu-P2*	TxRd-ACg.ACA.gCC.ATg.CAg.CAC.CT-BHQ2

❑ **Note**: If problems occur with competition using both Wu-probes, the use of just the Gram negative Wu Probe, Wu-P2 is permitted.

- qPCR Master Mix for a TaqMan assay (e.g. Applied TaqMan Universal Mastermix II)
- Real-time PCR machine (e.g. Bio-rad CFX96, Qiagen Rotorgene-Q)
- PCR grade 2 mL and 1.5 mL microtubes
- Qiagen DNeasy Plant Mini DNA extraction kit

3.3.2. Preparation of seed extracts and controls

- Use sterile techniques during the extraction procedure.
- Add each seed sample, PPC and NPC into 100 mL sSEB in a separate sterile container.
- As a Negative Extraction Control (NEC), add 100 mL sSEB in a sterile container (without seeds).
- As an IAC, add 100 µL of Acat, a boiled bacterial suspension provided by the organiser, to each of the 15 samples, PPC, NPC and NEC.
- Soak seed samples and controls and incubate overnight (16-18 hour) at 4-7 °C.

3.3.3. Bacteria extraction (SE-qPCR and dilution plating)

- After 16-18 hour soaking, shake the samples for 5 min at room temperature (20-25 °C) on an orbital shaker set at 150-200 rpm.
- For dilution plating:
  - Sample 2 mL seed extract from each seed sample, PPC and NPC and keep on ice until dilution plating **according to ISTA Rule 7-020.**
  - Proceed with ISTA Rule 7-020 from 2.2 to 6.6 and next to 3.3.6.
- For SE-qPCR:
  - Wear gloves and prevent cross contamination between samples and controls.
  - Sample 10 mL seed extract from each seed sample, PPC, NPC and NEC. Filter the extract using a sterile syringe and micropore membrane syringe filter (5 µm) and capture 5 mL filtered extract into a 10 mL tube.
  - Centrifuge the filtered seed extracts for 10 min at 5,300 x g.
  - Carefully decant the supernatant from each sub sample and keep the pellet. Be aware not to decant the pellet together with the supernatant.
  - Use the pellet for the isolation of DNA using the Qiagen Plant Mini Kit.
  - ❑ At this point it is also possible to use an in-house DNA extraction kit (King Fisher, Roche MP96, Macherey-Nagel etc.) in addition to the Qiagen Plant Mini Kit.
  - Proceed with step 3.3.4.

3.3.4. DNA Extraction (SE-qPCR)

- Disinfect the flow cabinet with 10% household chlorine solution and at least 30 min UV light.
- Add 400 µL Buffer AP1 and 4 µL RNase-A stock solution (100 mg/mL). Re-suspend the pellet, vortex and incubate at 65 °C for 10 min.
- Add 130 µL Buffer P3 to the lysate, mix, and incubate for 5 min on ice or at +4 °C.
- Centrifuge the lysate for 5 min at 20,000 RCF.
- Pipet the lysate into the QIA shredder Mini spin column (lilac) placed in a 2 mL collection tube, and centrifuge for 2 min at 20,000 RCF.
- Transfer the flow-through into a new tube without disturbing the cell-debris pellet.
- Add 1.5 volumes of Buffer AW1 to the cleared lysate, and mix by pipetting.

- Pipet 650 µL of the mixture, including any precipitate that may have formed, into the DNeasy Mini spin column placed in a 2 mL collection tube. Centrifuge for 1 min at 6,000 RCF and discard the flow-through.
- Add 500 µL Buffer AW2 and centrifuge for 1 min at 6,000 RCF. Discard the flow-through.
- Repeat the wash step by adding 500 µL Buffer AW2 to the DNeasy Mini spin column, and centrifuge for 2 min at 20,000 RCF to dry the membrane.
- Elute the extracted and purified DNA by adding 100 µL AE Buffer, incubate at room temperature for 5 min and centrifuge into a clean 1.5 mL tube at 6,000 RCF for 1 min.
- Proceed with step 3.3.5.

3.3.5. PCR assays for SE-qPCR

3.3.5.1. Reaction mixture and PCR conditions

Table A.1 provides an example for the reaction mixtures.

❑ **Note:** The reaction mixture and conditions need to be checked and/or optimized within each laboratory before starting the comparative test.

Table A.1a. Reaction mixture for duplex MVS Xhc-Acat qPCR.

	Duplex MVS-Xhc/Acat qPCR		
	[Stock]	Volume 1x (µL)	[Final]
qPCR Master Mix	2x	10.0	1x
MVSXhc-3F	20 µM	0.30	300 nM
MVSXhc-3R	20 µM	0.30	300 nM
MVSXhc-3P	20 µM	0.15	150 nM
Acat-2F	20 µM	0.20	200 nM
Acat-2R	20 µM	0.20	200 nM
Acat-1Pr	20 µM	0.10	100 nM
Nucleic acid-free water	1x	3.75	-
Total PCR mix	-	15.0	-
DNA sample	-	5.0	-
Total Volume	-	20.0	-

Table A.1b. Reaction mixture for duplex Temple Xhc-Acat qPCR.

	Duplex Temple-Xhc/Acat qPCR		
	[Stock]	Volume 1x (µL)	[Final]
qPCR Master Mix	2x	10.0	1x
Xhc-q2F	20 µM	0.30	300 nM
Xhc-q2R	20 µM	0.30	300 nM
Xhc-q2P	20 µM	0.15	150 nM
Acat-2F	20 µM	0.20	200 nM
Acat-2R	20 µM	0.20	200 nM
Acat-1Pr	20 µM	0.10	100 nM
Nucleic acid-free water	1x	3.75	-
Total PCR mix	-	15.0	-
DNA sample	-	5.0	-
Total Volume	-	20.0	-

- Test the DNA extracts from the seed samples, PPC, NPC and NEC with the duplex MVS-Xhc/Acat and the duplex Temple-Xhc/Acat.
- Also test PAC-Xhc-1, PAC-Xhc-2, PAC-Xhc-3, PAC-Acat and NTC with the duplex MVS-Xhc/Acat and the duplex Temple-Xhc/Acat.
- Prepare enough reaction mixture for both duplex qPCR assays (guidelines in Table A.1) to perform duplicate reactions for all samples.
- Minimise the exposure of the probes to light.
- Put 15.0 µL PCR reaction mixture in a 96-well PCR plate.
- Add in duplicate 5.0 µL DNA extract from the samples and all controls in corresponding wells.
- Cover the PCR plate (sealing).
- Centrifuge the plate for 1 min at 2.000g.
- Perform qPCR according to Table A.2.

❑ **Note**: The reaction mixture and conditions need to be checked and/or optimized within each laboratory before starting the comparative test.

Table A.2. PCR conditions for duplex or triplex Xhc qPCR.

Denaturation	10 min 95 °C
Denaturation/Elongation	40x : 15 sec 95 °C - 60 sec 60 °C

3.3.5.2. Interpretation of SE-qPCR results

The amplification curves will be analysed with a threshold fixed above the background fluorescence within the exponential amplification phase of the amplification curves.

The quantification cycle (Cq) will be used to identify positive reactions. True positive reactions show a typical exponential increase (S-curve) in fluorescence.

- Record **all the Cq values** in Appendix 1

– The **expected** PCR test results:

Suspect samples	detection of Xhc-DNA (Cq ⩽ 40) and detection of Acat-DNA (Cq ⩽ 35)
Suspect samples	detection of Xhc-DNA (Cq ⩽ 40) and no detection (due to competition) of Acat-DNA (Cq ⩾ 35)
Negative Samples	no detection of Xhc-DNA (Cq ⩾ 40), detection of Acat-DNA (Cq ⩽ 35)
NTC	no detection of Xhc-DNA and no detection of Acat-DNA (Cq ⩾ 40)
PPC	detection of Xhc-DNA (Cq ⩽ 40) and Acat-DNA (Cq ⩽ 35)
NPC	no detection of Xhc-DNA, detection of Acat-DNA (Cq ⩽ 35)
NEC	no detection of Xhc-DNA, detection of Acat-DNA (Cq ⩽ 35)
PAC-Xhc (1,2,3)	detection of Xhc-DNA (Cq ⩽ 35), no detection of Acat-DNA
PAC-Acat	no detection of Xhc-DNA, detection of Acat-DNA (Cq ⩽ 35)
IAC (PEC)	detection of Acat-DNA (Cq ⩽ 35)

3.3.6. Xhc colony confirmation (dilution plating assay)

3.3.6.1. qPCR assay for Xhc colony confirmation

Table A.3 provides an example for the reaction mixtures.

❑ **Note:** The reaction mixture and conditions need to be checked and/or optimized within each laboratory before starting the comparative test.

NOTE: This section replaces the sections 8.2-8.7 in ISTA Rule 7-020.

– Test the bacterial suspensions from suspected Xhc colonies as prepared in step 8.1 from ISTA Rule 7-020.

– **Store the remaining bacterial suspension of the suspected colonies at -20 °C. In case it is needed for validation, this suspension can be used for gel-based PCR as described in ISTA Rule 7-020**.

– Include a non-suspect colony and a suspect Xhc colony from the known Xhc strain as an extra Negative Process Control (NPC) and Positive Process Control (PPC) for the PCR colony confirmation.

– Also include PAC-Xhc-1, PAC-Xhc-2 and NTC.

– Wear gloves and prevent cross contamination between samples.

– Prepare enough reaction mixture (guidelines in Table 3) for the triplex qPCR assay.

– Minimise the exposure of the probes to light.

Table A.3. Reaction mixture for triplex MVS-Xhc/Temple-Xhc/Wu qPCR.

	Triplex MVS-Xhc/Temple-Xhc/Wu qPCR		
	[Stock]	Volume 1x (µL)	[Final]
qPCR Master Mix	2x	10.0	1x
MVSXhc-3F	20 µM	0.90	900 nM
MVSXhc-3R	20 µM	0.90	900 nM
MVSXhc-3P	20 µM	0.25	250 nM
Xhc-q2F	20 µM	0.40	400 nM
Xhc-q2R	20 µM	0.40	400 nM
Xhc-q2P	20 µM	0.20	200 nM
Wu-F	20 µM	0.20	200 nM
Wu-R	20 µM	0.20	200 nM
Wu-P1	20 µM	0.20	200 nM
Wu-P2	20 µM	0.20	200 nM
Nucleic acid-free water	1x	4.15	-
Total PCR mix	-	18.0	-
DNA sample	-	2.0	-
Total Volume	-	20.0	-

- Put 18.0 µL PCR reaction mixture in a 96-well PCR plate.
- Add 2.0 µL bacterial suspension, and controls in corresponding wells.
- Cover the PCR plate (sealing).
- Centrifuge the plate for 1 min at 2.000g.
- Perform qPCR according to Table A.2.

❑ **Note**: The reaction mixture and conditions need to be checked and/or optimised within each laboratory before starting the comparative test

- Record the results in Appendix 1.

3.3.6.2. Interpretation of the qPCR results

- It is known that the Wu-assay reacts with residual microbial DNA present in the polymerase in qPCR master mixes, which in some cases leads to the possibility of a rather low Cq-value of 28 for the NTC. To determine if a sample is properly prepared the Cq-value of the Wu assay in Xhc negative samples should be at least 3 Cq values lower than the NTC. In other words, the sample needs to contain almost 10 times more microbial DNA than is present in the NTC.
- When the difference in the Cq-value of the NTC and the sample is ⩽ 3, a new sample must be tested and compared with a new NTC.
- When the amount of DNA of the target pathogens is high, competition is observed in the pathogen-specific (Temple-Xhc and MVS-Xhc) and the Wu assays. In such cases the interpretation scheme presented in Table A.4 should be used.
- Record the results in Appendix 1

Table A.4. qPCR scoring interpretation scheme for Xhc colony confirmation.

Temple-Xhc qPCR	MVS-Xhc qPCR	Wu qPCR	Result[1]
Cq ⩽ 32	Not relevant	Not relevant	Xhc suspect
Not relevant	Cq ⩽ 32	Not relevant	Xhc suspect
Cq > 32	Cq > 32	Cq ⩽ Cq NTC-3	Xhc negative
Cq > 32	Cq > 32	Cq > Cq NTC-3	invalid

[1] Scores can only be given when the Cq-values for Wu-assay in the samples are at least 3 Cq values lower than that found in the NTC.

3.4 Critical points:

- On receiving seed samples, NPC, PPC: store below 15 °C (4 to 15 °C).
- NTC, PAC-Xhc (1-3), PAC-Acat and IAC must be stored at -20 °C immediately upon arrival
- Prevent DNA contamination.
- Store extracted DNA at -20 °C in case further analysis is required.
- Store bacterial suspensions of suspected colonies in case further analysis is required.
- Specify all results, PCR reaction mixtures and all other relevant information in Appendix 1.
- Do not deviate from the proposed method unless otherwise permitted in the protocol.
- Before starting the comparative test, get experience with the proposed method with provided practice samples. These have an infection rate equal or little over of a low positive seed lot. Perform the comparative test with persons experienced with seed health pre-screening based on molecular methods.

4. References

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Appendix 1. Template results file inter-laboratory comparative test.

Appendix 1.xlsx

Annex B. Summary results of the inter-laboratory comparative test obtained by each laboratory for all tested samples and controls.

NPC: negative process control, PPC: Positive process control. Positive deviation are indicated by yellow cells and negative deviation by blue cells.

Contamination level	Sample	Laboratory									
		A	B	C	D	E	F	G	H	I	J
CT_high	**4**	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CT_high	**5**	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CT_high	**9**	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CT_medium	**3**	pos	pos	neg	pos	pos	pos	pos	pos	pos	pos
CT_medium	**6**	pos	pos	pos	pos	neg	neg	pos	pos	pos	pos
CT_medium	**7**	pos	neg	neg	pos	pos	pos	neg	pos	pos	pos
CT_medium	**8**	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CT_medium	**11**	pos	neg	pos	neg	pos	pos	pos	pos	pos	pos
CT_medium	**12**	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CT_medium	**13**	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CT_medium	**14**	pos	neg	pos	pos	pos	pos	pos	pos	pos	pos
CT_medium	**15**	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos
CT_healthy	**1**	neg	neg	neg	neg	neg	neg	pos	neg	neg	neg
CT_healthy	**2**	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
CT_healthy	**10**	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg
NPC	**NPC**	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
PPC	**PPC**	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos

Annex C. Raw results of the inter-laboratory comparative test obtained by each laboratory for all tested samples and controls.

For each laboratory there are two tables. the first table has the number of colonies recorded and the second table has the Cq values obtained for the qPCR identification. C: confluent, so much growth that no separate colonies are visible anymore, M: many colonies, but still visible as separate colonies, ND: not countable (> 150 suspected Xhc colonies or plating default), NPC: negative process control, NTC: negative template control, PAC: positive amplification control, PPC: positive process control, SAP: overgrown by saprophytes, TNTC: colonies too numerous to count.

Annex C Cont.

Lab. A – Table 1		MKM medium							mTBM medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
1	0x	0	TNTC	0	0	0	0	0	0	93	0	0	0	0	0
1	10x	0	87	0	0	0	0	0	0	19	0	0	0	0	0
1	100x	0	20	0	0	0	0	0	0	1	0	0	0	0	0
1	1000x	0	4	0	0	0	0	0	0	0	0	0	0	0	0
2	0x	3	TNTC	3	2	1	1	0	1	TNTC	1	1	0	0	0
2	10x	1	105	1	1	1	1	0	0	30	0	0	0	0	0
2	100x	0	24	0	0	0	0	0	0	4	0	0	0	0	0
2	1000x	0	3	0	0	0	0	0	0	0	0	0	0	0	0
3	0x	73	19	6	6	1	1	1	98	0	7	6	1	1	1
3	10x	8	5	6	6	0	0	0	35	2	6	6	2	2	2
3	100x	1	0	1	1	1	1	1	7	1	6	6	1	1	1
3	1000x	0	0	0	0	0	0	0	1	0	1	1	1	1	1
4	0x	112	68	7	4	1	1	1	210	0	7	5	1	1	1
4	10x	50	31	6	5	0	0	0	67	0	6	5	2	2	2
4	100x	9	10	6	5	0	0	0	8	1	6	6	2	2	2
4	1000x	0	0	0	0	0	0	0	0	1	0	0	0	0	0
5	0x	TNTC	21	6	6	2	2	2	TNTC	2	6	5	0	0	0
5	10x	112	12	6	6	2	2	2	152	3	6	6	0	0	0
5	100x	17	6	6	6	1	1	1	25	0	6	6	1	1	1
5	1000x	3	0	3	3	1	1	1	0	4	0	0	0	0	0
6	0x	5	42	5	5	2	2	2	6	39	6	6	2	2	2
6	10x	0	6	0	0	0	0	0	5	3	5	4	2	2	2
6	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0x	64	58	6	6	1	1	1	128	0	6	6	1	1	1

Annex C Cont.

Lab. A – Table 1		MKM medium							mTBM medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
7	10x	9	6	6	6	1	1	1	31	3	6	6	1	1	1
7	100x	4	0	4	4	2	2	2	0	0	0	0	0	0	0
7	1000x	1	0	1	1	0	0	0	0	0	0	0	0	0	0
8	0x	81	27	6	4	1	1	1	132	21	6	4	1	1	1
8	10x	13	0	6	6	2	2	2	15	2	6	4	0	0	0
8	100x	1	0	1	1	1	1	1	4	0	4	3	1	1	1
8	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0x	86	63	6	5	1	1	1	127	4	6	3	1	1	1
9	10x	23	20	6	4	2	2	2	44	0	6	5	0	0	0
9	100x	2	1	2	2	1	1	1	4	0	4	4	0	0	0
9	1000x	0	0	0	0	0	0	0	3	0	3	2	1	1	1
10	0x	3	79	3	1	1	1	0	4	128	4	2	0	0	0
10	10x	4	42	4	2	2	2	1	0	38	0	0	0	0	0
10	100x	1	26	1	1	1	1	0	1	2	1	0	0	0	0
10	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0x	9	62	6	3	3	3	3	15	73	6	3	1	1	1
11	10x	1	16	1	1	1	1	1	2	22	2	1	1	1	1
11	100x	0	2	0	0	0	0	0	0	0	0	0	0	0	0
11	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0x	9	48	6	4	2	2	2	16	3	6	4	1	1	1
12	10x	1	6	1	1	1	1	1	5	32	5	2	2	2	2
12	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0x	0	53	0	0	0	0	0	4	56	4	4	4	4	4
13	10x	0	4	0	0	0	0	0	2	6	2	2	2	2	2

Annex C Cont.

Lab. A – Table 1		MKM medium							mTBM medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
13	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0x	72	18	6	6	1	1	1	168	12	6	3	0	0	0
14	10x	24	4	6	6	2	2	2	30	2	6	3	0	0	0
14	100x	1	0	1	1	1	1	1	3	7	3	1	1	1	1
14	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0x	12	72	6	5	1	1	1	22	29	6	5	1	1	1
15	10x	2	27	2	2	1	1	1	5	5	5	5	1	1	1
15	100x	2	2	2	2	2	2	2	0	0	0	0	0	0	0
15	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	0x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	10x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PPC	0x	TNTC	0	2	2	1	1	1	TNTC	0	2	2	1	1	1
PPC	10x	138	0	2	2	1	1	1	165	0	2	2	1	1	1
PPC	100x	14	0	2	2	1	1	1	24	0	2	2	1	1	1
PPC	1000x	3	0	2	2	1	1	1	3	0	2	2	1	1	1
NPC	0x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NPC	10x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NPC	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NPC	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Annex C. Cont.

Lab. A – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
1	2	MKM	0x	>40	>40	14.48	negative
2	2	MKM	10x	>40	>40	13.23	negative
3	3	MKM	0x	16.08	16	14.1	suspect
4	3	mTBM	0x	15.78	15.77	14.18	suspect
5	3	mTBM	10x	15.6	15.77	13.83	suspect
6	3	mTBM	10x	16.14	16	14.08	suspect
7	3	MKM	100x	16.2	16.13	14.4	suspect
8	3	mTBM	100x	15.72	15.76	14.21	suspect
9	3	mTBM	1000x	15.72	15.88	13.91	suspect
10	4	MKM	0x	15.62	15.68	13.78	suspect
11	4	mTBM	0x	15.68	15.68	13.82	suspect
12	4	mTBM	10x	15.76	15.87	13.76	suspect
13	4	mTBM	10x	22.35	22.32	14.9	suspect
14	4	mTBM	100x	15.4	15.34	13.72	suspect
15	4	mTBM	100x	15.14	14.93	13.23	suspect
16	5	MKM	0x	24.99	25.21	14.99	suspect
17	5	MKM	0x	20.46	20.68	14.94	suspect
18	5	MKM	10x	15.23	15.05	13.56	suspect
19	5	MKM	10x	15.93	15.85	14.44	suspect
20	5	MKM	100x	15.69	15.67	14.02	suspect
21	5	mTBM	100x	15.73	15.75	14.37	suspect
22	5	MKM	1000x	15.51	15.39	13.97	suspect
23	6	MKM	0x	16.28	16.19	14.51	suspect
24	6	MKM	0x	20.13	20.3	15.16	suspect
25	6	mTBM	0x	14.79	14.8	12.91	suspect
26	6	mTBM	0x	15.63	15.51	13.78	suspect
27	6	mTBM	10x	1606	15.92	14.07	suspect
28	6	mTBM	10x	15.97	15.75	13.99	suspect
29	7	MKM	0x	15.9	15.98	14.3	suspect
30	7	mTBM	0x	15.98	16.01	14.32	suspect
31	7	MKM	10x	15.51	15.39	13.88	suspect
32	7	mTBM	10x	15.91	15.91	14.73	suspect
33	7	MKM	100x	15.51	15.55	14.42	suspect
34	7	MKM	100x	15.75	15.71	14.39	suspect
35	8	MKM	0x	15.28	15.08	13.54	suspect
36	8	mTBM	0x	15.84	15.79	13.93	suspect
37	8	MKM	10x	15.26	15.18	13.44	suspect
38	8	MKM	10x	15.37	15.28	13.67	suspect
39	8	MKM	100x	14.99	14.85	13.07	suspect
40	8	mTBM	100x	15.54	15.42	13.64	suspect
41	9	MKM	0x	17.38	17.42	15.56	suspect
42	9	mTBM	0x	23.27	23.54	14.97	suspect
43	9	MKM	10x	16.28	16.24	14.71	suspect
44	9	MKM	10x	15.47	15.44	13.72	suspect
45	9	mTBM	100x	16.36	16.31	14.77	suspect

Annex C Cont.

Lab. A – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
46	9	mTBM	1000x	16.36	16.32	14.63	suspect
47	10	MKM	0x	>40	>40	14.31	negative
48	10	MKM	10x	20.94	20.86	16.85	suspect
49	10	MKM	10x	>40	>40	16.25	negative
50	10	MKM	100x	>40	>40	15.08	negative
51	11	MKM	0x	16.16	16.04	14.45	suspect
52	11	MKM	0x	16.5	16.4	14.87	suspect
53	11	MKM	0x	16.09	16.06	14.48	suspect
54	11	mTBM	0x	16.51	16.48	14.95	suspect
55	11	MKM	10x	16.4	16.39	14.71	suspect
56	11	mTBM	10x	16.3	16.26	14.71	suspect
57	12	MKM	0x	15.59	15.53	13.93	suspect
58	12	MKM	0x	15.1	14.99	13.57	suspect
59	12	mTBM	0x	15.68	15.63	14.1	suspect
60	12	MKM	10x	15.67	15.66	13.94	suspect
61	12	mTBM	10x	15.61	15.58	13.71	suspect
62	12	mTBM	10x	16.07	15.95	14.31	suspect
63	13	mTBM	0x	16.6	16.3	14.63	suspect
64	13	mTBM	0x	16.45	16.19	14.5	suspect
65	13	mTBM	0x	16.12	15.97	14.32	suspect
66	13	mTBM	0x	16.52	16.27	14.5	suspect
67	13	mTBM	0x	37.02	16.45	14.77	suspect
68	13	mTBM	0x	15.75	15.83	13.92	suspect
69	14	MKM	0x	13.83	13.83	12.07	suspect
70	14	MKM	10x	14.15	13.61	11.75	suspect
71	14	MKM	10x	15.07	14.91	13.1	suspect
72	14	MKM	100x	16.08	15.9	13.98	suspect
73	14	mTBM	100x	15.7	15.71	13.81	suspect
74	15	MKM	0x	15.94	15.98	14.01	suspect
75	15	mTBM	0x	16.62	16.49	14.67	suspect
76	15	MKM	10x	16.97	16.96	15.2	suspect
77	15	mTBM	10x	15.84	15.82	14.14	suspect
78	15	MKM	100x	15.59	15.58	13.78	suspect
79	15	MKM	100x	15.72	17.73	13.92	suspect
80	PPC	MKM	0x	15.03	15.19	14.65	suspect
81	PPC	mTBM	0x	15.99	16.11	15.66	suspect
82	PPC	MKM	10x	14.91	14.98	13.95	suspect
83	PPC	mTBM	10x	14.7	14.21	12.93	suspect
84	PPC	MKM	100x	14.21	13.95	12.13	suspect
85	PPC	mTBM	100x	15.46	15.14	13.38	suspect
86	PPC	MKM	1000x	15.66	15.48	13.67	suspect
87	PPC	mTBM	1000x	15.72	15.14	13.45	suspect
88	NPC (Xcc)			>40	>40	14.97	negative
89	NPC (Cmm)			>40	>40	16.16	negative

Annex C Cont.

Lab. A – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
PAC-Xhc-1				21.54	21.3	19.48	suspect
PAC-Xhc-2				21.94	21.95	20.54	suspect
NTC-organiser	water			38.19	>40	33.65	negative
NTC-ours	water			>40	>40	33.92	negative

NTC-organiser: used the organiser provided water
NTC-ours: Used our sterile water.

Annex C. Cont.

Lab. B – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
1	0x	0	TNTC	0					0	TNTC	0				
1	10x	0	80	0					3	TNTC	3	0	3	1-3	0
1	100x	0	18	0					0	67	0				
1	1000x	0	1	0					0	10	0				
2	0x	0	TNTC	0					0	TNTC	0				
2	10x	0	88	0					1	90	1	0	1	4	0
2	100x	0	27	0					1	30	1	1	1	5	0
2	1000x	0	5	0					0	9	0				
3	0x	8	11	3	3	3	6-8	3	3	50	3	3	3	9-11	3
3	10x	0	1	0					0	7	0				
3	100x	0	0	0					0	0	0				
3	1000x	0	0	0					0	0	0				
4	0x	240	0	0					120	100	0				
4	10x	31	0	2	2	2	12-13	2	30	15	2	2	2	16-17	2
4	100x	3	0	1	1	1	14	1	5	0	1	1	1	18	1
4	1000x	1	0	1	1	1	15	1	1	0	1	1	1	19	1
5	0x	TNTC	0	0					TNTC	TNTC	0				
5	10x	78	0	2	2	2	20-21	2	40	48	2	2	2	24-25	2
5	100x	14	0	1	1	1	22	1	7	5	1	1	1	26	1
5	1000x	3	0	1	1	1	23	1	6	0	1	1	1	27	1
6	0x	60	9	2	2	2	28-29	2	65	48	2	2	2	32-33	2
6	10x	7	1	2	2	2	30-31	2	4	10	2	2	2	34-35	2
6	100x	0	0	0					0	2	0				
6	1000x	0	0	0					0	2	0				

Annex C Cont.

Lab. B – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
7	0x	0	8	0					0	21	0				
7	10x	0	1	0					0	2	0				
7	100x	0	0	0					0	0	0				
7	1000x	0	0	0					0	0	0				
8	0x	5	24	2	2	2	36-37	2	7	68	3	3	3	39-41	2
8	10x	1	1	1	1	1	38	1	0	10	0				
8	100x	0	0	0					0	0	0				
8	1000x	0	0	0					0	0	0				
9	0x	TNTC	0	1	1	1	42	1	TNTC	TNTC	1	1	1	46	1
9	10x	50	0	2	2	2	43-44	2	56	46	2	2	2	47-48	2
9	100x	5	0	1	1	1	45	1	7	6	1	1	1	49	1
9	1000x	0	0	0					0	2	0				
10	0x	0	TNTC	0					0	TNTC	0				
10	10x	0	80	0					3	TNTC	3	0	3	50-52	0
10	100x	0	18	0					0	56	0				
10	1000x	0	6	0					0	12	0				
11	0x	0	9	0					0	52	0				
11	10x	0	0	0					0	5	0				
11	100x	0	0	0					0	0	0				
11	1000x	0	0	0					0	0	0				
12	0x	3	13	2	2	2	53-54	2	3	22	2	2	2	56-57	2
12	10x	0	2	1	1	1	55	1	1	1	1	1	1	58	1
12	100x	1	0	0					0	0	0				
12	1000x	0	0	0					0	0	0				

Annex C Cont.

Lab. B – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
13	0x	25	20	2	2	2	59-60	2	5	45	2	2	2	62-63	2
13	10x	2	0	1	1	1	61	1	1	50	1	1	1	64	1
13	100x	0	0	0					0	0	0				
13	1000x	0	0	0					0	0	0				
14	0x	0	32	0					0	35	0				
14	10x	0	2	0					0	5	0				
14	100x	0	1	0					0	1	0				
14	1000x	0	0	0					0	1	0				
15	0x	42	8	2	2	2	65-66	2	23	25	2	2	2	68-69	2
15	10x	6	3	1	1	1	67	1	2	3	1	1	1	70	1
15	100x	0	0	0					0	0	0				
15	1000x	0	0	0					0	0	0				
sterility check	0x	0	0	0					0	0	0				
sterility check	10x	0	0	0					0	0	0				
sterility check	100x	0	0	0					0	0	0				
sterility check	1000x	0	0	0					0	0	0				
PPC	0x	280	1	1	1	1	71	1	200	50	1	1	1	75	1
PPC	10x	73	0	2	2	2	72-73	2	50	30	2	2	2	76-77	2
PPC	100x	11	0	1	1	1	74	1	3	3	1	1	1	78	1
PPC	1000x	0	0	0					3	0	0				
NPC	0x	0	TNTC	0	0				0	TNTC	0	0			
NPC	10x	0	120	0	0	1	79	0	0	TNTC	0	0	1	81	0
NPC	100x	0	36	0	0	1	80	0	0	52	0	0	1	82	0
NPC	1000x	0	3	0	0				0	12	0	0			

Annex C. Cont.

Lab. B – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
1	1	MD5A	10x	>40	>40	24.99	negative
2	1	MD5A	10x	>40	>40	24.25	negative
3	1	MD5A	10x	>40	>40	24.17	negative
4	2	MD5A	10x	>40	>40	18.13	negative
5	2	MD5A	100x	>40	>40	21.45	negative
6	3	MKM	0x	>40	17.41	17.53	positive
7	3	MKM	0x	>40	17.37	17.79	positive
8	3	MKM	0x	>40	17.10	17.12	positive
9	3	MD5A	0x	>40	17.21	17.53	positive
10	3	MD5A	0x	15.78	16.25	17.12	positive
11	3	MD5A	0x	>40	16.09	23.06	positive
12	4	MKM	10x	16.48	18.03	19.40	positive
13	4	MKM	10x	17.06	18.06	18.36	positive
14	4	MKM	100x	15.39	16.46	21.73	positive
15	4	MKM	1000x	15.54	16.27	18.13	positive
16	4	MD5A	10x	15.40	16.32	17.64	positive
17	4	MD5A	10x	15.34	16.59	19.70	positive
18	4	MD5A	100x	15.96	16.62	17.06	positive
19	4	MD5A	1000x	15.19	15.80	19.27	positive
20	5	MKM	10x	14.78	15.62	21.64	positive
21	5	MKM	10x	15.06	16.08	17.51	positive
22	5	MKM	100x	14.76	15.99	18.38	positive
23	5	MKM	1000x	16.07	17.12	17.87	positive
24	5	MD5A	10x	15.03	16.25	24.95	positive
25	5	MD5A	10x	15.52	16.76	26.14	positive
26	5	MD5A	100x	14.90	15.70	22.44	positive
27	5	MD5A	1000x	14.77	16.35	-	positive
28	6	MKM	10x	15.09	16.27	18.69	positive
29	6	MKM	10x	15.33	16.27	17.33	positive
30	6	MKM	100x	15.65	16.20	16.90	positive
31	6	MKM	1000x	14.88	15.59	18.98	positive
32	6	MD5A	10x	15.44	16.35	17.37	positive
33	6	MD5A	10x	14.55	15.85	23.58	positive
34	6	MD5A	100x	14.74	15.25	19.74	positive
35	6	MD5A	1000x	14.73	15.19	20.36	positive
36	8	MKM	0x	15.19	16.42	18.55	positive
37	8	MKM	0x	16.10	16.51	16.92	positive
38	8	MKM	10x	17.13	18.27	18.66	positive
39	8	MD5A	0x	17.25	18.66	20.39	positive
40	8	MD5A	0x	15.46	16.42	17.67	positive
41	8	MD5A	0x	17.01	18.57	19.29	positive
42	9	MKM	0x	15.10	16.04	18.02	positive
43	9	MKM	10x	15.75	16.74	17.75	positive
44	9	MKM	10x	15.78	17.00	17.65	positive
45	9	MKM	100x	16.01	18.05	20.51	positive
46	9	MD5A	0x	15.29	16.72	18.73	positive

Annex C Cont.

Lab. B – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
47	9	MD5A	10x	15.59	16.38	16.90	positive
48	9	MD5A	10x	14.66	15.64	22.16	positive
49	9	MD5A	100x	15.41	16.50	18.59	positive
50	10	MD5A	10x	>40	>40	21.60	negative
51	10	MD5A	10x	>40	>40	19.49	negative
52	10	MD5A	10x	>40	>40	23.03	negative
53	12	MKM	0x	14.81	16.18	20.64	positive
54	12	MKM	0x	14.85	15.89	21.71	positive
55	12	MKM	10x	14.96	16.12	20.72	positive
56	12	MD5A	0x	15.17	16.04	17.12	positive
57	12	MD5A	0x	13.78	18.79	-	positive
58	12	MD5A	10x	15.04	16.02	18.05	positive
59	13	MKM	0x	14.87	15.43	18.65	positive
60	13	MKM	0x	14.96	15.71	17.89	positive
61	13	MKM	10x	14.58	15.81	22.25	positive
62	13	MD5A	0x	16.13	17.06	18.49	positive
63	13	MD5A	0x	14.55	15.72	20.11	positive
64	13	MD5A	10x	14.82	15.76	19.40	positive
65	15	MKM	0x	15.43	16.14	17.32	positive
66	15	MKM	0x	14.82	15.71	18.91	positive
67	15	MKM	10x	14.97	15.97	18.96	positive
68	15	MD5A	0x	14.64	16.06	20.25	positive
69	15	MD5A	0x	15.14	15.96	18.38	positive
70	15	MD5A	10x	15.29	16.19	17.24	positive
71	PPC	MKM	10x	13.78	15.02	-	positive
72	PPC	MKM	10x	14.16	15.69	26.47	positive
73	PPC	MKM	100x	14.47	15.44	20.65	positive
74	PPC	MKM	1000x	14.39	15.18	22.99	positive
75	PPC	MD5A	10x	14.63	15.41	19.60	positive
76	PPC	MD5A	10x	14.78	16.31	20.48	positive
77	PPC	MD5A	100x	14.31	15.19	26.69	positive
78	PPC	MD5A	1000x	13.81	14.48	30.64	positive
79	NPC	MKM	10x	>40	>40	18.59	negative
80	NPC	MKM	100x	>40	>40	17.37	negative
81	NPC	MD5A	10x	>40	>40	18.23	negative
82	NPC	MD5A	100x	>40	>40	17.63	negative
PAC-Xhc-1				20.72	21.16	20.18	positive
PAC-Xhx-2				23.24	23.80	22.54	positive
PAC-Xhx-3				33.44	33.61	32.12	positive
NTC				-	-	34.88	negative

Annex C. Cont.

Lab. C – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
1	0x	0?	C						0?	C					
1	10x	0	M						0	M					
1	100x	0	76/107						0	81/88					
1	1000x	0	5/10						0	11/6					
2	0x	0?	C						0?	C					
2	10x	0	M						0	M					
2	100x	0	50/51						0	45/49					
2	1000x	0	9/13						0	4/8					
3	0x	1/0	57/58	1/0	0				0	69/57					
3	10x	0	11/8						0	7/14					
3	100x	0	1/0						0	2/0					
3	1000x	0	0						0	0/1					
4	0x	M	M						M	M					
4	10x	75/70	28/35						29/49	60/69					
4	100x	3/4	1/1	1/4	1/4	x/1	2	1	3/4	3/11	3/3	2/3	1/1	3, 4	2
4	1000x	1/0	0	1/0	1/x	1/x	1	1	0	0/1					
5	0x	M	M						M	M					
5	10x	48/75	29/27						13/18	10/10	4/0	4/x			
5	100x	5/4	5/6	3/3	3/3	1/1	5, 6	2	0/1	0	0/1	x/1	x/1	7	1
5	1000x	0	0/1						0/1	0	0/1	x/1	x/1	8	1
6	0x	87/64	24/31						15/15	35/36	3/0	3/x			
6	10x	13/7	5/10	6/0	6/x	2/x	9, 10	2	1/0	6/1	1/0	1/x	1/x	12	1
6	100x	1/0	1/0						2/0	0	2/0	2/x	1/x	11	1
6	1000x	0	0						0	0					

Annex C Cont.

Lab. C – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
7	0x	0	16/17						11*/1*	20/25	6/0	0			
7	10x	0	3/2						0	2/3					
7	100x	0	0						0	0/1					
7	1000x	0	0						0	0					
8	0x	260/268	24/17						100/130	55/51					
8	10x	34/27	2/3						8/10	3/4	0/6	x/6	x/2	15, 16	2
8	100x	4/5	0	3/3	3/3	1/1	13, 14	2	0	0/1					
8	1000x	0	1/0						0	0					
9	0x	C	M						C	M					
9	10x	197/197	68/67						161/177	70/54					
9	100x	22/28	10/5	0/6	x/6	x/2	17, 18	2	25/25	11/8					
9	1000x	3/2	0						2/7	0/3	0/6	x/6	x/2	19, 20	2
10	0x	0?	C						0?	C					
10	10x	0	M						0	M					
10	100x	0	58/47						0	41/52					
10	1000x	0	6/11						0	4/2					
11	0x	153/175	M						75/44	142/160					
11	10x	17/28	16/26						2/4	26/29	2/2	2/2			
11	100x	3/3	0	3/3	3/3	1/1	21, 22	2	1/1	4/1	1/1	1/1	1/1	23, 24	2
11	1000x	1/0	0						0	0/1					
12	0x	2/0	69/50	2/0	2/x	2/x	25, 26	2	2/4	77/72	2/4	0/0			
12	10x	0	5/5						0	9/5					
12	100x	0	0/1						0	4/1					
12	1000x	0	0						0	0					

Annex C Cont.

Lab. C – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
13	0x	4/6	65/65	4/0	4/x	1/x	28	1	4/6	69/81	3/3	2/2	1/1	29, 30	2
13	10x	1/1	9/7	1/1	1/1	1/x	27	1	0	6/6					
13	100x	0	0						0	1/0					
13	1000x	0	0						0	0					
14	0x	4/9	M	3/0	3/x				3/6	M	0/6	x/3	x/2	33, 34	2
14	10x	0/1	46/38	0/1	x/1				0	55/42					
14	100x	1/1	1/5	1/1	1/1	1/1	31, 32	2	0	5/1					
14	1000x	0	0/1						0	0/1					
15	0x	20/21	129/131	0/2	x/2				7/9	137/121	3/3	3/3	1/1	37, 38	2
15	10x	3/0	17/21	3/0	3/x	1/x	36	1	0	28/21					
15	100x	1/0	0/2	1/0	1/x	1/x	35	1	0	0/1					
15	1000x	0	0						0	0/1					
sterility check	0x	0	0						0	0					
sterility check	10x	0	0						0	0					
sterility check	100x	0	0						0	0					
sterility check	1000x	0	0						0	0					
PPC	0x	197/156	M						80/70	C					
PPC	10x	26/29	56/62	1/0	1/x				16/14	86/120					
PPC	100x	1/4	5/4	1/4	1/4	1/1	39, 40	2	3/3	9/10	2/3	2/3	1/x	42	1
PPC	1000x	0	0/1						1/0	0/1	1/0	1/x	1/x	41	1
NPC	0x	0?	C						0?	C					
NPC	10x	0	M						0	M					
NPC	100x	0	33/55						0	34/55					
NPC	1000x	0	8/4						0	5/7					

Annex C. Cont.

Lab. C – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
1	4	MKM	1000x	19.69	21.35	18.84	positive
2	4	MKM	100x	19.35	21.06	18.35	positive
3	4	MD5A	100x	21.21	23.03	20.93	positive
4	4	MD5A	100x	20.09	21.94	19.80	positive
5	5	MKM	100x	23.46	25.11	23.06	positive
6	5	MKM	100x	21.91	23.60	21.40	positive
7	5	MD5A	100x	21.43	23.08	20.93	positive
8	5	MD5A	1000x	20.33	21.91	19.50	positive
9	6	MKM	10x	22.01	23.81	21.89	positive
10	6	MKM	10x	21.07	22.82	20.87	positive
11	6	MD5A	100x	22.13	24.01	21.62	positive
12	6	MD5A	10x	22.17	24.16	21.58	positive
13	8	MKM	100x	22.14	23.72	21.22	positive
14	8	MKM	100x	20.03	21.66	19.48	positive
15	8	MD5A	10x	20.38	21.96	19.70	positive
16	8	MD5A	10x	19.87	21.57	18.78	positive
17	9	MKM	100x	22.65	24.40	22.06	positive
18	9	MKM	100x	21.51	23.17	20.63	positive
19	9	MD5A	1000x	21.06	22.88	19.92	positive
20	9	MD5A	1000x	20.68	22.44	20.42	positive
21	11	MKM	100x	21.03	22.63	20.58	positive
22	11	MKM	100x	20.35	21.93	20.07	positive
23	11	MD5A	100x	22.00	23.56	21.41	positive
24	11	MD5A	100x	20.65	22.22	19.75	positive
25	12	MKM	0x	21.20	22.86	20.60	positive
26	12	MKM	0x	23.41	25.21	23.01	positive
27	13	MKM	10x	20.12	21.79	19.52	positive
28	13	MKM	0x	21.52	23.34	20.85	positive
29	13	MD5A	0x	21.52	23.15	21.01	positive
30	13	MD5A	0x	20.03	21.90	19.96	positive
31	14	MKM	10x	20.74	22.56	20.51	positive
32	14	MKM	100x	21.11	22.94	20.79	positive
33	14	MD5A	0x	22.80	24.72	22.23	positive
34	14	MD5A	0x	22.06	24.02	21.60	positive
35	15	MKM	100x	22.05	23.83	21.79	positive
36	15	MKM	10x	21.54	23.30	21.27	positive
37	15	MD5A	0x	21.47	23.38	20.70	positive
38	15	MD5A	0x	21.90	23.74	21.76	positive
39	PPC	MKM	100x	23.62	25.46	23.31	positive
40	PPC	MKM	100x	22.97	24.82	22.57	positive
41	PPC	MD5A	1000x	21.66	23.53	21.23	positive
42	PPC	MD5A	100x	22.01	23.84	21.53	positive
PPC				22.45	24.30	22.33	positive
NPC				-	-	19.80	negative
PAC-Xhc-1				19.41	21.12	-	OK
PAC-Xhc-2				21.47	23.36	21.10	OK

Annex C Cont.

Lab. C – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
NTC				-	-	34.64	OK

Annex C. Cont.

Lab. D – Table 1		MKM medium							MD5A or mTBM medium (specify)						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
1	0x	0	200	0	0	0	0	0	1	500	1	0	0	0	0
1	10x	1	50	1	1	1	308-1a	0	2	50	2	2	2	308-1a/b	0
1	100x	1	10	1	1	1	308.2b	0	0	10	0	0	0	0	0
1	1000x	0	1	0	0	0	0	0	0	2	0	0	0	0	0
2	0x	0	200	0	0	0	310-1	0	0	500	0	0	0	0	0
2	10x	1	30	1	1	1	0	0	1	50	1	1	1	310-1	0
2	100x	0	10	0	0	0	0	0	0	10	0	0	0	0	0
2	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0x	22	6	2	2	2	312-0a/b	2	28	5	1	1	1	312-0	1
3	10x	1	0	1	1	1	312-1	1	5	1	2	2	2	312-1a/b	2
3	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0x	TNTC	0	1	1	1	314-0	1	TNTC	10	0	0	0	0	0
4	10x	81	0	1	1	1	314-1	1	110	10	1	1	1	314-1	1
4	100x	5	0	1	1	1	314-2	1	12	2	1	1	1	314-2	1
4	1000x	1	0	0	0	0	0	0	1	0	1	1	1	314-3	1
5	0x	TNTC	SAP+INHIB?	0	0	0	0	0	TNTC	SAP+INHIB?	0	0	0	0	0
5	10x	65	10	1	1	1	316-1	1	104	20	1	1	1	316-1	1
5	100x	9	1	1	1	1	316-2	1	15	5	1	1	1	316-2	1
5	1000x	1	0	1	1	1	316-3	1	2	0	1	1	1	316-3	1
6	0x	9	SAP+INHIB?	1	1	1	318-0	1	1	5	2	2	2	318-0a/b	2
6	10x	1	0	1	1	1	318-1	1	1	0	1	1	1	318-1	1
6	100x	1	0	1	1	1	318-2	1	0	0	0	0	0	0	0
6	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Annex C Cont.

Lab. D – Table 1		MKM medium							MD5A or mTBM medium (specify)						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
7	0x	7	5	1	1	1	320-0	1	6	1	2	2	2	320-0a/b	2
7	10x	1	0	1	1	1	320-1	1	1	0	1	1	1	320-1	1
7	100x	1	0	1	1	1	320-2	1	0	0	0	0	0	0	0
7	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0x	0	5	0	0	0	0	0	1	10	1	1	1	322-0	1
8	10x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0x	TNTC	200	0	0	0	0	0	TNTC	500	0	0	0	0	0
9	10x	85	10	1	1	1	324-1	1	65	20	1	1	1	324-1	1
9	100x	14	2	1	1	1	324-2	1	10	5	1	1	1	324-2	1
9	1000x	1	0	1	1	1	324-3	1	2	0	1	1	1	324-3	1
10	0x	0	500	0	0	0	0	0	0	500	0	0	0	0	0
10	10x	0	100	0	0	0	0	0	0	100	0	0	0	0	0
10	100x	0	20	0	0	0	0	0	0	20	0	0	0	0	0
10	1000x	0	1	0	0	0	0	0	0	2	0	0	0	0	0
11	0x	0	10	0	0	0	0	0	0	5	0	0	0	0	0
11	10x	0	2	0	0	0	0	0	1	1	1	0	0	0	0
11	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0x	0	10	0	0	0	0	0	1	100	1	1	1	330-0	1
12	10x	0	0	0	0	0	0	0	0	5	0	0	0	0	0
12	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Annex C Cont.

Lab. D – Table 1		MKM medium							MD5A or mTBM medium (specify)						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
13	0x	2	5	2	2	2	332-0b/c	2	4	5	2	2	2	332-0a/b	2
13	10x	0	0	0	0	0	0	0	1	1	1	1	1	332-1	1
13	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	1000x	0	1	0	0	0	0	0	0	0	0	0	0	0	0
14	0x	0	5	3	3	0	0	0	3	1	3	3	3	334-0a/b/c	3
14	10x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0x	0	10	0	0	0	0	0	1	10	1	1	1	336-0	1
15	10x	0	1	0	0	0	0	0	0	0	0	0	0	0	0
15	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	0x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	10x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PPC	0x	TNTC	0	1	1	1	338-0	1	TNTC	50	0	0	0	0	0
PPC	10x	36	0	1	1	1	338-1	1	85	10	1	1	1	338-1	1
PPC	100x	3	0	1	1	1	338-2	1	5	0	1	1	1	338-2	1
PPC	1000x	0	0	0	0	0	0	0	1	0	1	1	1	338-3	1
NPC	0x	0	500	0	0	0	0	0	0	500	0	0	0	0	0
NPC	10x	0	100	0	0	0	0	0	0	100	0	0	0	0	0
NPC	100x	0	20	0	0	0	0	0	0	10	0	0	0	0	0

Annex C Cont.

Lab. D – Table 1		MKM medium							MD5A or mTBM medium (specify)						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
NPC	1000x	0	2	0	0	0	0	0	0	1	0	0	0	0	0

Annex C. Cont.

Lab. D – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
308-1a	1	MKM	10x	Undetermined	Undetermined	18.03	negative
308-2b	1	MKM	100x	Undetermined	Undetermined	16.78	negative
308-1a	1	mTBM	10x	Undetermined	Undetermined	18.12	negative
308-1b	1	mTBM	10x	Undetermined	Undetermined	18.67	negative
310-1	2	MKM	10x	Undetermined	Undetermined	16.76	negative
312-0a	3	MKM	x	19.51	17.9	17.28	positive
312-0b	3	MKM	x	19.23	17.52	17.38	positive
312-1	3	MKM	10x	19.79	17.36	16.92	positive
312-0	3	mTBM	x	18.46	16.01	15.97	positive
312-1a	3	mTBM	10x	19.42	16.84	17.32	positive
312-1b	3	mTBM	10x	19.7	17.04	17.48	positive
314-0	4	MKM	x	20.17	17.15	17.58	positive
314-1	4	MKM	10x	20.03	18.47	18.09	positive
314-2	4	MKM	100x	19.69	17.27	17.78	positive
314-1	4	mTBM	10x	19.91	17.69	18.03	positive
314-2	4	mTBM	100x	18.31	15.96	15.98	positive
314-3	4	mTBM	1000x	21.11	18.69	18.79	positive
316-1	5	MKM	10x	19.7	17.34	17.17	positive
316-2	5	MKM	100x	19.34	17.81	17.83	positive
316-3	5	MKM	1000x	19.62	17.54	17.45	positive
316-1	5	mTBM	10x	19.52	17.31	17.2	positive
316-2	5	mTBM	100x	18.86	16.27	16.12	positive
316-3	5	mTBM	1000x	20.07	17.52	17.55	positive
318-0	6	MKM	x	19.41	16.81	16.75	positive
318-1	6	MKM	10x	19.22	17.73	17.8	positive
318-2	6	MKM	100x	19.35	17.17	17.37	positive
318-0a	6	mTBM	x	20.6	17.59	17.85	positive
318-0b	6	mTBM	x	19.99	17.61	17.65	positive
318-1	6	mTBM	10x	18.93	16.61	16.64	positive
320-0	7	MKM	x	21.15	18.55	18.4	positive
320-1	7	MKM	10x	19.76	17.8	17.53	positive
320-2	7	MKM	100x	19.83	17.13	17.38	positive
320-0a	7	mTBM	x	20.56	18	18.53	positive
320-0b	7	mTBM	x	19.32	16.66	16.77	positive
320-1	7	mTBM	10x	20.29	17.8	17.65	positive
322-0	8	mTBM	x	19.05	16.78	16.89	positive
324-1	9	MKM	10x	19.64	18.31	18.13	positive
324-2	9	MKM	100x	18.75	16.99	16.95	positive
324-3	9	MKM	1000x	20.3	18.78	18.6	positive
324-1	9	mTBM	10x	21.14	19.5	19.51	positive
324-2	9	mTBM	100x	20.76	18.89	19.42	positive
324-3	9	mTBM	1000x	19.12	17.24	17.67	positive
330-0a	12	mTBM	x	19.7	17.83	17.99	positive
332-0b	13	MKM	x	18.84	17.32	17.24	positive
332-0c	13	MKM	x	19.84	18.28	18.21	positive
332-0a	13	mTBM	x	19.97	17.75	17.98	positive

Annex C Cont.

Lab. D – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
332-0b	13	mTBM	x	21.81	19.54	19.85	positive
332-1	13	mTBM	10x	19.47	17.39	18.1	positive
334-0a	14	mTBM	x	19.7	17.22	17.91	positive
334-0b	14	mTBM	x	18.78	16.57	17.13	positive
334-0c	14	mTBM	x	20.81	18.86	18.86	positive
336-0	15	mTBM	x	18.91	16.68	17.12	positive
338-0	PPC	MKM	x	20.51	18.32	18.82	positive
338-1	PPC	MKM	10x	18.85	16.58	16.96	positive
338-2	PPC	MKM	100x	17.81	15.4	15.95	positive
338-1	PPC	mTBM	10x	18.17	15.61	16.09	positive
338-2	PPC	mTBM	100x	18.27	16.48	16.63	positive
338-3	PPC	mTBM	1000x	19.36	16.7	17.52	positive
T+ Xhc	PPC	x	x	21.18	18.61	19.41	positive
T- Ps tomato	NPC	x	x	Undetermined	Undetermined	16.52	negative
Water	NTC	x	x	Undetermined	Undetermined	23.87	negative
PAC-Xhc-1	x	x	x	19.93	17.71	17.75	positive
PAC-Xhc-2	x	x	x	22.9	21.17	20.48	positive
NTC	x	x	x	Undetermined	Undetermined	23.98	negative

Annex C. Cont.

Lab. E – Table 1		MKM medium							MD5A or mTBM medium (specify)						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
1	0x	0	Dirty TNTC	0	0	0			0	Dirty TNTC	0	0	0		
1	10x	0	Dirty TNTC	0	0	0			0	TNTC	0	0	0		
1	100x	0	200	0	0	0			1?	TNTC	1	1	1	1	0
1	1000x	0	120	0	0	0			0	50	0	0	0		
2	0x	0	Dirty TNTC	0	0	0			0	Dirty TNTC	0	0	0		
2	10x	0	Dirty TNTC	0	0	0			0	Dirty TNTC	0	0	0		
2	100x	0	300	0	0	0			0	150	0	0	0		
2	1000x	0	50	0	0	0			0	14	0	0	0		
3	0x	? Fungal contamination	? Fungal Contamination	0	0	0			3	TNTC (1 plate fungal)	3	3	2	4,5	2
3	10x	4	20	4	4	2	2,3	2	1	41	1	1	0		
3	100x	0	5	0	0	0			0	3	0	0	0		
3	1000x	0	16	0	0	0			0	0	0	0	0		
4	0x	~200	TNTC (1 plate fungal)	0	0	0			250	TNTC (1 plate fungal)	0	0	0		
4	10x	36	50	4	4	2	6,7	2	46	TNTC	4	4	2	8,9	2
4	100x	4	8	0	0	0			6	50	0	0	0		
4	1000x	0	3	0	0	0			1	0	0	0	0		
5	0x	TNTC	TNTC fungal Dirty	0	0	0			TNTC	TNTC	0	0	0		
5	10x	95	75	4	4	2	12,13	2	175	100	4	4	2	10,11	2

Annex C Cont.

Lab. E – Table 1		MKM medium							MD5A or mTBM medium (specify)						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
5	100x	18	25	0	0	0			18	7	0	0	0		
5	1000x	1	3	0	0	0			3	3	0	0	0		
6	0x	0	200	0	0	0			0	TNTC	0	0	0		
6	10x	0	50	0	0	0			0	79	0	0	0		
6	100x	0	9	0	0	0			0	17	0	0	0		
6	1000x	0	0	0	0	0			0	0	0	0	0		
7	0x	154	50 w/fungal	0	0	0			270	TNTC (1 plate fungal)	0	0	0		
7	10x	21	5	4	4	2	16,17	2	15	25	4	4	2	14,15	2
7	100x	3	0	0	0	0			5	2	0	0	0		
7	1000x	0	~300	0	0	0			0	0	0	0	0		
8	0x	1	50	1	1	1	18	1	1	20	1	1	1	19	1
8	10x	0	5	0	0	0			0	9	0	0	0		
8	100x	0	0	0	0	0			0	0	0	0	0		
8	1000x	0	0	0	0	0			0	0	0	0	0		
9	0x	TNTC	TNTC Dirty	0	0	0			TNTC	TNTC Dirty	0	0	0		
9	10x	~200	60	0	0	0			200	TNTC (1 plate fungal)	0	0	0		
9	100x	13	5	4	4	2	22,23	2	23	7	4	4	2	20,21	2
9	1000x	2	2	0	0	0			6	1	0	0	0		
10	0x	?	TNTC Dirty	0	0	0			0	TNTC Dirty	0	0	0		

Annex C Cont.

Lab. E – Table 1		MKM medium							MD5A or mTBM medium (specify)						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
10	10x	0	300	0	0	0			0	TNTC (1 plate fungal)	0	0	0		
10	100x	0	100	0	0	0			0	40	0	0	0		
10	1000x	0	25	0	0	0			0	10	0	0	0		
11	0x	8	40	4	4	2	24,25	2	28	75 (1 plate fungal)	4	4	2	26,27	2
11	10x	2	6	2	0	0			2	4	0	0	0		
11	100x	0	7	0	0	0			0	0	0	0	0		
11	1000x	0	0	0	0	0			0	0	0	0	0		
12	0x	70	75	4	4	2,4	30,31 56,57,58, 59	4	28	50 (1 plate fungal)	4	4	2,4	28,29 60,61,62, 63	4
12	10x	2	21	2	2	0			3	24	0	0	0		
12	100x	0	6	0	0	0			0	4	0	0	0		
12	1000x	0	TNTC Fungal	0	0	0			0	5	0	0	0		
13	0x	38	100	0	0	0			35	200	4	4	2	32,33	2
13	10x	12	5	4	4	2	34,35	2	3	9	0	0	0		
13	100x	0	0	0	0	0			0	0	0	0	0		
13	1000x	0	0	0	0	0			0	0	0	0	0		
14	0x	56	100	0	0	0			115	100	0	0	0		
14	10x	23	13	4	4	2	36,37	2	13	12	4	4	2	38,39	2
14	100x	0	4	0	0	0			1	4	0	0	0		
14	1000x	0	1	0	0	0			1	0	0	0	0		

Annex C Cont.

Lab. E – Table 1		MKM medium							MD5A or mTBM medium (specify)						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
15	0x	3	100	3	3	3	40,41,43	3	2	200	2	2	2	44,45	2
15	10x	0	11	0	0	0			0	32	0	0	0		
15	100x	0	6	0	0	0			0	7	0	0	0		
15	1000x	0	0	0	0	0			0	1	0	0	0		
sterility check	0x	0	0	0	0	0			0	0	0	0	0		
sterility check	10x	0	0	0	0	0			0	0	0	0	0		
sterility check	100x	0	0	0	0	0			0	0	0	0	0		
sterility check	1000x	0	0	0	0	0			0	0	0	0	0		
PPC	0x	TNTC	75	0	0	0			TNTC	TNTC	0	0	0		
PPC	10x	79	6	4	4	2	49,50	2	89	54	4	4	2	51,52	2
PPC	100x	8	2	0	0	0			8	5 (1 fungal plate)	0	0	0		
PPC	1000x	1	1	0	0	0			4	1	0	0	0		
NPC	0x	?	TNTC Dirty	0	0	0			2?	TNTC Dirty	2?	2?	2?	46,47	0
NPC	10x	0	300	0	0	0			1?	300	1?	1?	1?	48	0
NPC	100x	1?	50	1?	1?	1?	45	0	0	67	0	0	0		
NPC	1000x	0	32	0	0	0			0	11	0	0	0		

Annex C. Cont.

Lab. E – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
1	1	TBM	100x	27.99	26.78	18.66	negative
2	3	MKM	10X	20.78	19.345	15.99	positive
3	3	MKM	10X	20.99	19.575	16.045	positive
4	3	TBM	0X	21.52	20.24	16.745	positive
5	3	TBM	0X	20.925	19.555	16.13	positive
6	4	MKM	10X	21.25	19.875	16.47	positive
7	4	MKM	10X	21.525	20.08	16.57	positive
8	4	TBM	10X	21.425	19.97	16.525	positive
9	4	TBM	10X	22.165	20.83	17.585	positive
10	5	TBM	10X	20.53	19.26	15.865	positive
11	5	TBM	10X	22.315	20.965	17.745	positive
12	5	MKM	10X	22.07	20.71	17.25	positive
13	5	MKM	10X	21.435	19.97	16.495	positive
14	7	TBM	10X	22.385	21.135	17.885	positive
15	7	TBM	10X	21.795	20.515	17.075	positive
16	7	MKM	10X	22.4	21.02	17.775	positive
17	7	MKM	10X	21.875	20.54	17.12	positive
18	8	KM	0x	22.11	20.65	17.425	positive
19	8	TBM	0x	21.985	20.54	17.32	positive
20	9	TBM	100x	20.865	19.51	16.135	positive
21	9	TBM	100x	21.915	20.665	17.24	positive
22	9	MKM	100x	21.74	20.335	17.04	positive
23	9	MKM	100x	21.535	20.105	16.785	positive
24	11	MKM	0x	21.675	20.235	16.835	positive
25	11	MKM	0x	21.335	19.93	16.355	positive
26	11	TBM	0x	22.15	20.65	17.345	positive
27	11	TBM	0x	22.025	20.59	17.385	positive
28	12	TBM	0x	28.15	20.11	17.165	positive
29	12	TBM	0x	21.81	20.26	17.01	positive
30	12	MKM	0x	28.09	20.97	17.995	positive
31	12	MKM	0x	28.055	20.79	17.66	positive
32	13	TBM	0x	25.01	23.69	21.425	positive
33	13	TBM	0x	21.955	20.415	17.205	positive
34	13	MKM	10x	21.74	20.305	16.975	positive
35	13	MKM	10x	22.245	20.7	17.52	positive
36	14	MKM	10x	21.555	20.07	16.865	positive
37	14	MKM	10x	21.75	20.385	17.05	positive
38	14	TBM	10x	22.365	20.845	17.605	positive
39	14	TBM	10x	21.05	19.545	16.26	positive
40	15	MKM	0x	22.01	20.52	17.43	positive
41	15	MKM	0x	22.01	20.67	17.56	positive
42	15	MKM	0x	22.28	20.915	17.76	positive
43	15	TBM	0x	22.18	20.82	17.64	positive
44	15	TBM	0x	21.655	20.325	17.07	positive
45	NPC	MKM	100x	27.975	26.71	18.885	negative
46	NPC	TBM	0x	27.915	26.655	19.525	negative

Annex C Cont.

Lab. E – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
47	NPC	TBM	0x	27.94	26.735	18.45	negative
48	NPC	TBM	10x	27.805	26.575	19.355	negative
49	PPC	MKM	10x	22.39	20.97	17.82	positive
50	PPC	MKM	10x	22.215	20.92	17.54	positive
51	PPC	TBM	10x	21.855	20.36	17.24	positive
52	PPC	TBM	10x	22.845	21.66	18.61	positive
53	NTC-Barnstead Water			28.03	26.78	25.67	negative
54	NTC-Nucleause Free Water			27.405	26.04	24.655	negative
55	Positive Control			21.885	20.595	17.485	positive

Annex C. Cont.

Lab. F – Table 1		MKM medium							MD5A or mTBM medium (specify)						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
1	0x	0	>300	0					10	>300	6	5	5	41-45	0
1	10x	0	~100	0					3	~150	0				
1	100x	0	15	0					0	32	0				
1	1000x	0	2	0					0	2	0				
2	0x	0	>300	0					0	>300	0				
2	10x	0	~75	0					0	~100	0				
2	100x	0	10	0					0	35	0				
2	1000x	0	0	0					0	3	0				
3	0x	1	26	1	1	1	1	1	9	92	3	2	2	49-50	2
3	10x	0	10	0					3	7	3	3	3	46-48	3
3	100x	0	0	0					0	2	0				
3	1000x	0	0	0					0	0	0				
4	0x	194	0	3	3	3	2-4	3	~120	13	2	2	2	55-56	2
4	10x	15	0	0					26	0	2	2	2	51-52	2
4	100x	3	0	3	3	3	5-7	3	4	0	2	2	2	53-54	2
4	1000x	0	0	0					0	0	0				
5	0x	5	~150	5	1	1	8	1	45	~200	2	2	2	57-58	2
5	10x	0	0						4	~100	2	2	2	59-60	2
5	100x	0	1	0					2	8	2	1	1	61	1
5	1000x	0	0	0					1	1	1	1	1	62	1
6	0x	0	~80	0					0	~100	0				
6	10x	0	16	0					0	13	0				
6	100x	0	1	0					0	1	0				
6	1000x	0	0	0					0	0	0				

Annex C Cont.

Lab. F – Table 1		MKM medium							MD5A or mTBM medium (specify)						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
7	0x	3	5	3	2	2	9-10	2	2	54	2	2	2	63-64	2
7	10x	0	0	0					1	4	1	1	1	65	1
7	100x	0	0	0					0	1	0				
7	1000x	0	0	0					0	0	0				
8	0x	11	5	5	3	3	11-13	3	7	67	4	4	4	66-69	4
8	10x	1	0	1	1	1	14	1	2	4	2	2	2	70-71	2
8	100x	0	0	0					0	0	0				
8	1000x	0	0	0					0	0	0				
9	0x	~50	0	2	2	2	15-16	2	~80	~150	2	2	2	72-73	2
9	10x	10	0	2	2	2	17-18	2	18	65	2	2	2	74-75	2
9	100x	2	0	2	2	2	19-20	2	3	5	2	2	2	76-77	2
9	1000x	0	0	0	0	0			0	0	0				
10	0x	0	>300	0					0	~200	0				
10	10x	0	~50	0					0	~100	0				
10	100x	0	12	0					0	20	0				
10	1000x	0	2	0					0	1	0				
11	0x	2	9	2	2	2	21-22	2	6	~45	6	5	5	78-82	5
11	10x	0	0	0					0	2	0				
11	100x	0	0	0					0	0	0				
11	1000x	0	0	0					0	0	0				
12	0x	4	4	4	4	4	23-26	4	4	25	4	2	2	83-84	2
12	10x	1	3	1					0	5	0				
12	100x	0	0	0					0	0	0				
12	1000x	0	0	0					0	0	0				

Annex C Cont.

Lab. F – Table 1		MKM medium							MD5A or mTBM medium (specify)						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
13	0x	0	~65	0					3	~65	3	3	3	85-87	2
13	10x	0	2	0					0	10	0				
13	100x	0	0	0					0	1	0				
13	1000x	0	0	0					0	0	0				
14	0x	1	4	1	1	1	28	1	20	15	5	5	5	88-92	5
14	10x	2	1	2	1	1	27	1	1	0	1	1	1	93	1
14	100x	0	0	0					0	0	0				
14	1000x	0	0	0					0	0	0				
15	0x	10	7	6	6	6	29-34	6	15	~165	3	3	3	94-96	2
15	10x	0	0	0					4	20	2	1	1	97	1
15	100x	0	0	0					1	0	1	1	1	98	1
15	1000x	0	0	0					0	0	0				
sterility check	0x	0	0	0					0	0	0				
sterility check	10x	0	0	0					0	0	0				
sterility check	100x	0	0	0					0	0	0				
sterility check	1000x	0	0	0					0	0	0				
PPC	0x	25	4	3	3	3	35-37	3	~150	~100	0				
PPC	10x	3	0	3	3	3	38-40	3	19	28	0				
PPC	100x	0	0	0					3	2	0				
PPC	1000x	0	0	0					1	1	0				
NPC	0x	0	>300						0	>200	0				
NPC	10x	1	50	1					0	84	0				
NPC	100x	0	15	0					0	24	0				
NPC	1000x	0	3						0	5	0				

Annex C. Cont.

Lab. F – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
1	3	MKM	0x	17.21	17.4	N/A	positive
2	4	MKM	0x	17.96	18.01	N/A	positive
3	4	MKM	0x	17.85	17.72	N/A	positive
4	4	MKM	0x	17.87	17.55	N/A	positive
5	4 100x	MKM	100x	16.87	16.86	N/A	positive
6	4 100x	MKM	100x	18.86	18.85	N/A	positive
7	4 100x	MKM	100x	18.68	18.58	N/A	positive
8	5	MKM	0x	19.42	19.39	N/A	positive
9	7	MKM	0x	17.16	17.26	N/A	positive
10	7	MKM	0x	17.8	17.49	N/A	positive
11	8 10x	MKM	10x	18.31	18.26	N/A	positive
12	8	MKM	0x	17.08	17.21	N/A	positive
13	8	MKM	0x	18.5	18.47	N/A	positive
14	8	MKM	0x	17.87	18.03	N/A	positive
15	9	MKM	0x	17.09	17.21	N/A	positive
16	9	MKM	0x	18.86	18.76	N/A	positive
17	9 10x	MKM	10x	18.12	18.13	N/A	positive
18	9 10x	MKM	10x	17.59	17.54	N/A	positive
19	9 100x	MKM	100x	19.02	19.15	N/A	positive
20	9 100x	MKM	100x	17.7	17.64	N/A	positive
21	11	MKM	0x	16.58	16.27	N/A	positive
22	11	MKM	0x	17.56	17.33	N/A	positive
23	12	MKM	0x	16.44	16.24	N/A	positive
24	12	MKM	0x	17.17	17.28	N/A	positive
25	12	MKM	0x	18.71	18.58	N/A	positive
26	12	MKM	0x	18.58	18.59	N/A	positive
27	14 10x	MKM	10x	17.56	17.38	N/A	positive
28	14	MKM	0x	16.93	16.88	N/A	positive
29	15	MKM	0x	16.85	16.86	N/A	positive
30	15	MKM	0x	17.09	17.26	N/A	positive
31	15	MKM	0x	17.44	17.18	N/A	positive
32	15	MKM	0x	17.96	18.13	N/A	positive
33	15	MKM	0x	16.47	16.4	N/A	positive
34	15	MKM	0x	18.55	18.51	N/A	positive
35	PPC	MKM	0x	16.43	16.2	N/A	positive
36	PPC	MKM	0x	17.63	17.37	N/A	positive
37	PPC	MKM	0x	15.29	15.18	N/A	positive
38	PPC	MKM	0x	17.84	17.61	N/A	positive
39	PPC	MKM	0x	18.58	18.58	N/A	positive
40	PPC	MKM	0x	18.58	18.39	N/A	positive
41	1	MD5A	0x	N/A	N/A	30.79	negative
42	1	MD5A	0x	N/A	N/A	21.8	negative
43	1	MD5A	0x	N/A	N/A	29.12	negative
44	1	MD5A	0x	N/A	N/A	23.17	negative
45	1	MD5A	0x	N/A	N/A	29.42	negative

Annex C Cont.

Lab. F – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
46	3 10x	MD5A	10x	17.96	17.95	N/A	positive
47	3 10x	MD5A	10x	19.59	19.55	N/A	positive
48	3 10x	MD5A	10x	18.27	18.29	N/A	positive
49	3	MD5A	0x	18.93	18.71	N/A	positive
50	3	MD5A	0x	17.87	17.79	N/A	positive
51	4 10x	MD5A	10x	17.38	17.12	N/A	positive
52	4 10x	MD5A	10x	18.73	18.41	N/A	positive
53	4 100x	MD5A	100x	18.06	17.86	N/A	positive
54	4 100x	MD5A	100x	18.2	18.13	N/A	positive
55	4	MD5A	0x	18.38	18.39	N/A	positive
56	4	MD5A	0x	19.1	19.03	N/A	positive
57	5	MD5A	0x	18.14	18.04	N/A	positive
58	5	MD5A	0x	17.81	17.47	N/A	positive
59	5 10x	MD5A	10x	18.25	18.1	N/A	positive
60	5 10x	MD5A	10x	17.71	17.57	N/A	positive
61	5 100x	MD5A	100x	19.02	19.02	N/A	positive
62	5 1000x	MD5A	1000x	18.01	17.81	N/A	positive
63	7	MD5A	0x	17.97	17.71	N/A	positive
64	7	MD5A	0x	19.81	19.76	N/A	positive
65	7 10x	MD5A	10x	18.03	17.9	N/A	positive
66	8	MD5A	0x	17.25	17.12	N/A	positive
67	8	MD5A	0x	18.45	18.48	N/A	positive
68	8	MD5A	0x	18.29	18.33	N/A	positive
69	8	MD5A	0x	17.86	17.58	N/A	positive
70	8 10x	MD5A	10x	18.17	18.22	N/A	positive
71	8 10x	MD5A	10x	18.31	18.33	N/A	positive
72	9	MD5A	0x	18.23	18.24	N/A	positive
73	9	MD5A	0x	17.25	17.15	N/A	positive
74	9 10x	MD5A	10x	17.52	17.48	N/A	positive
75	9 10x	MD5A	10x	16.52	16.43	N/A	positive
76	9 100x	MD5A	100x	16.89	16.72	N/A	positive
77	9 100x	MD5A	100x	18.12	18.02	N/A	positive
78	11	MD5A	0x	18.67	18.4	N/A	positive
79	11	MD5A	0x	18.03	17.92	N/A	positive
80	11	MD5A	0x	20.02	19.92	N/A	positive
81	11	MD5A	0x	19.35	19.44	N/A	positive
82	11	MD5A	0x	18.84	18.68	N/A	positive
83	12	MD5A	0x	17.04	16.97	N/A	positive
84	12	MD5A	0x	19.05	18.98	N/A	positive
85	13	MD5A	0x	19.13	19.13	N/A	positive
86	13	MD5A	0x	18.12	18.04	N/A	positive
87	13	MD5A	0x	N/A	N/A	20.69	negative
88	14	MD5A	0x	18.62	18.54	N/A	positive
89	14	MD5A	0x	18.22	18.34	N/A	positive
90	14	MD5A	0x	18.13	18.05	N/A	positive

Annex C Cont.

Lab. F – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
91	14	MD5A	0x	18.77	18.61	N/A	positive
92	14	MD5A	0x	19.25	19.21	N/A	positive
93	14 10x	MD5A	10x	17.03	16.85	N/A	positive
94	15	MD5A	0x	N/A	N/A	37.76	ITD
95	15	MD5A	0x	17.2	17.15	N/A	positive
96	15	MD5A	0x	18.63	18.51	N/A	positive
97	15 10x	MD5A	10x	17.92	17.68	N/A	positive
98	15 100x	MD5A	100x	17.75	17.54	N/A	positive
PPC				16.66	16.58	N/A	
NPC				N/A	N/A	16.54	
PAC-Xhc-1				19.39	19.41	N/A	
PAC-Xhc-2				19.52	19.81	N/A	
NTC				N/A	N/A	36.66	

Annex C. Cont.

Lab. G – Table 1		MKM medium							mTBM medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
1	0x	SAP	SAP						SAP	SAP					
1	10x	SAP	SAP						SAP	SAP-1	1	1	1		0
1	100x	1	0	1	1	1		1	2	0	2	2	2		0
1	1000x	0	0						0	0					
2	0x	SAP	SAP						SAP	SAP					
2	10x	SAP	SAP						0	0					
2	100x	SAP	SAP						0	0					
2	1000x	0	0						0	0					
3	0x	4	SAP	4	4	4		4	9	5	5	5	5		5
3	10x	1	0	1	1	1		1	0	0					
3	100x	0	0						0	0					
3	1000x	0	0						0	0					
4	0x	SAP	SAP						SAP	SAP					
4	10x	94	ND	2	2	2		2	69	61	2	2	2		2
4	100x	48	65	2	2	2		2	12	9	2	2	2		2
4	1000x	21	0	1	1	1		1	2	0	1	1	1		1
5	0x	ND	ND						SAP	SAP					
5	10x	46	59	3	3	3		3	67	26	2	2	2		2
5	100x	3	16	2	2	2		2	3	3	2	2	2		2
5	1000x	0	0						0	2	1	1	1		1
6	0x	7	4						SAP	13	2	2	2		2
6	10x	0	2	2	2	2		2	1	1	2	2	2		2
6	100x	0	0	2	2	2		2	0	1	1	1	1		1
6	1000x	1	0	1	1	1		1	0	0					

Annex C Cont.

Lab. G – Table 1		MKM medium							mTBM medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
7	0x	SAP	SAP						SAP	SAP					
7	10x	0	0						0	0					
7	100x	0	0						0	0					
7	1000x	0	0						0	0					
8	0x	12	SAP	1	1	1		1	SAP	SAP					
8	10x	2	2	2	2	2		2	5	6	4	4	4		4
8	100x	1	1	2	2	2		2	0	1	1	1	1		1
8	1000x	0	0						0	0					
9	0x	ND	ND						ND	ND					
9	10x	166	131	2	2	2		2	126	113	1	1	1		1
9	100x	17	ND	2	2	2		2	19	19	2	2	2		2
9	1000x	1	0	1	1	1		1	4	4	2	2	2		2
10	0x	SAP	SAP						SAP	SAP					
10	10x	SAP	SAP						0	0					
10	100x	SAP	SAP						0	0					
10	1000x	0	0						0	0					
11	0x	3	1	4	4	4		4	2	2	4	4	4		4
11	10x	1	0	1	1	1		1	0	0					
11	100x	0	0						0	0					
11	1000x	0	0						0	0					
12	0x	71	51	2	2	2		2	125	133	1	1	1		1
12	10x	6	5	2	2	2		2	18	7	2	2	2		2
12	100x	1	0	1	1	1		1	2	1	2	2	2		2
12	1000x	0	0						0	0					

Annex C Cont.

Lab. G – Table 1		MKM medium							mTBM medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
13	0x	1	2	3	3	3		2	SAP	3	3	3	3		3
13	10x	0	1	1	1	1		1	0	1	1	1	1		1
13	100x	1	0	1	1	1		1	0	0					
13	1000x	0	0						0	0					
14	0x	SAP-17	SAP-7	3	3	3		3	14	6	2	2	2		2
14	10x	1	1	2	2	2		2	1	2	2	2	2		2
14	100x	0	0						1	0	1	1	1		1
14	1000x	0	0						0	0					
15	0x	SAP	3	3	3	3		3	7	8	3	3	3		3
15	10x	0	1	1	1	1		1	1	1	2	2	2		2
15	100x	0	0						0	0					
15	1000x	0	0						0	0					
sterility check	0x	X	X						X	X					
sterility check	10x	X	X						X	X					
sterility check	100x	X	X						X	X					
sterility check	1000x	X	X						X	X					
PPC	0x	ND	ND						ND	ND					
PPC	10x	ND	ND						ND	ND					
PPC	100x	42	86	3	3	3		3	27	23	2	2	2		2
PPC	1000x	14	6	2	2	2		2	2	5	3	3	3		3
NPC	0x	SAP	SAP						SAP	SAP	2	2	2		0
NPC	10x	SAP	SAP	5	5	5		0	SAP	SAP	2	2	2		0
NPC	100x	SAP	SAP						0	0					
NPC	1000x	0	0						0	0					

Annex C. Cont.

Lab. G – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
1	1	mTBM	10x	-	-	9.97	negative
2	1	MKM	100x	31.22	29.44	17.53	positive
3	1	mTBM	100x	-	-	12.72	negative
4	1	mTBM	100x	-	-	12.57	negative
5	3	MKM	0x	19.84	16.46	17.2	positive
6	3	mTBM	0x	15.18	12.54	12.86	positive
7	3	MKM	0x	20.11	16.53	17.24	positive
8	3	mTBM	0x	15.45	12.94	13.1	positive
9	3	MKM	0x	20.38	16.55	17.33	positive
10	3	mTBM	0x	15.8	13.32	13.4	positive
11	3	MKM	0x	20.14	16.62	17.35	positive
12	3	mTBM	0x	15.44	13.39	13.4	positive
13	3	mTBM	0x	15.25	12.65	12.88	positive
14	3	MKM	10x	20.24	16.61	17.24	positive
15	4	MKM	10x	20.64	17.01	17.44	positive
16	4	mTBM	10x	14.75	12.23	12.51	positive
17	4	MKM	10x	19.75	16.18	16.79	positive
18	4	mTBM	10x	14.53	12.2	12.7	positive
19	4	MKM	100x	19.75	16.3	16.82	positive
20	4	mTBM	100x	15.06	12.59	12.67	positive
21	4	MKM	100x	19.79	16.26	16.99	positive
22	4	mTBM	100x	15.27	12.68	12.73	positive
23	4	MKM	1000x	20.27	16.55	17.24	positive
24	4	mTBM	1000x	14.98	12.62	12.65	positive
25	5	MKM	10x	19.94	16.27	16.99	positive
26	5	mTBM	10x	15.04	12.33	12.57	positive
27	5	MKM	10x	20.55	16.89	17.37	positive
28	5	mTBM	10x	14.43	12.33	12.5	positive
29	5	MKM	10x	20.19	16.55	17.21	positive
30	5	MKM	100x	20.05	16.54	17.13	positive
31	5	mTBM	100x	14.95	12.79	12.76	positive
32	5	MKM	100x	20.01	16.26	16.94	positive
33	5	mTBM	100x	15.56	13.24	13.21	positive
34	5	mTBM	1000x	15.49	12.8	12.97	positive
35	6	MKM	0x	20.26	16.77	17.48	positive
36	6	mTBM	0x	14.79	12.64	12.98	positive
37	6	MKM	0x	20.58	16.95	17.43	positive
38	6	mTBM	0x	14.86	12.41	12.6	positive
39	6	MKM	10x	19.76	16.23	16.9	positive
40	6	mTBM	10x	14.38	11.98	12.65	positive
41	6	MKM	10x	19.64	15.89	16.75	positive
42	6	mTBM	10x	14.83	12.13	12.63	positive
43	6	mTBM	100x	14.28	12.2	12.74	positive
44	6	MKM	1000x	20	16.2	16.86	positive
45	8	MKM	0x	19.52	16.01	16.56	positive
46	8	MKM	10x	19.62	16.18	16.78	positive

Annex C Cont.

Lab. G – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
47	8	mTBM	10x	15.24	12.45	12.69	positive
48	8	MKM	10x	20.52	16.76	17.28	positive
49	8	mTBM	10x	14.69	12.45	12.57	positive
50	8	mTBM	10x	14.69	12.35	12.51	positive
51	8	mTBM	10x	14.77	12.41	12.82	positive
52	8	mTBM	100x	14.78	12.32	12.6	positive
53	8	MKM	100x	19.34	15.8	16.61	positive
54	8	MKM	100x	19.75	16.15	16.74	positive
55	9	mTBM	10x	15.1	12.61	12.89	positive
56	9	MKM	10x	19.53	16.02	16.76	positive
57	9	MKM	10x	19.82	16.08	16.82	positive
58	9	MKM	100x	19.27	15.81	16.56	positive
59	9	mTBM	100x	14.32	11.78	12.22	positive
60	9	MKM	100x	19.4	15.93	16.61	positive
61	9	mTBM	100x	14.41	12.06	12.34	positive
62	9	MKM	1000x	19.46	15.92	16.82	positive
63	9	mTBM	1000x	14.54	12.01	12.33	positive
64	9	mTBM	1000x	14.48	12.26	12.46	positive
65	11	MKM	0x	20.1	16.46	17.11	positive
66	11	mTBM	0x	14.22	11.72	12.06	positive
67	11	MKM	0x	19.47	15.88	16.59	positive
68	11	mTBM	0x	14.73	12.33	12.54	positive
69	11	MKM	0x	19.97	16.3	17.01	positive
70	11	mTBM	0x	15.34	12.43	12.77	positive
71	11	MKM	0x	20.25	16.57	17.15	positive
72	11	mTBM	0x	14.77	12.28	12.61	positive
73	11	MKM	10x	20.07	16.32	17.02	positive
74	12	mTBM	0x	15.28	12.66	12.72	positive
75	12	MKM	0x	19.99	16.37	17.07	positive
76	12	MKM	0x	20.24	16.6	17.16	positive
77	12	MKM	10x	20.08	16.48	17.1	positive
78	12	mTBM	10x	14.98	12.66	12.76	positive
79	12	MKM	10x	20.41	16.56	17.06	positive
80	12	mTBM	10x	14.94	12.76	12.8	positive
81	12	MKM	100x	20.35	16.76	17.34	positive
82	12	mTBM	100x	14.36	11.84	12.25	positive
83	12	mTBM	100x	15.04	12.45	12.64	positive
84	13	mTBM	10x	15.19	12.52	12.57	positive
85	13	MKM	0x	20.38	16.97	17.37	positive
86	13	mTBM	0x	14.54	12.21	12.45	positive
87	13	MKM	0x	-	-	20.79	negative
88	13	mTBM	0x	14.85	12.24	12.49	positive
89	13	MKM	0x	20.32	16.7	17.3	positive
90	13	mTBM	0x	14.76	12.46	12.75	positive
91	13	MKM	10x	19.76	16.38	16.97	positive
92	13	MKM	100x	20.44	16.9	17.39	positive

Annex C Cont.

Lab. G – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
93	14	MKM	0x	19.97	16.5	17.07	positive
94	14	mTBM	0x	14.86	12.87	12.89	positive
95	14	MKM	0x	20.19	16.54	17.2	positive
96	14	mTBM	0x	15.06	12.52	12.69	positive
97	14	MKM	0x	19.71	16.22	16.83	positive
98	14	MKM	10x	19.87	16.45	16.99	positive
99	14	mTBM	10x	14.91	12.56	12.64	positive
100	14	MKM	10x	19.92	16.45	17.13	positive
101	14	mTBM	10x	15.1	12.73	12.79	positive
102	14	mTBM	100x	14.34	11.99	12.23	positive
103	15	MKM	0x	20.58	16.83	17.3	positive
104	15	mTBM	0x	15.56	12.76	12.9	positive
105	15	MKM	0x	19.77	16.15	16.79	positive
106	15	mTBM	0x	14.81	12.49	12.49	positive
107	15	MKM	0x	20.09	16.33	16.91	positive
108	15	mTBM	0x	15.01	12.51	12.6	positive
109	15	MKM	10x	19.85	16.25	16.87	positive
110	15	mTBM	10x	14.96	12.46	12.57	positive
111	15	mTBM	10x	14.86	12.38	12.44	positive
112	NPC	mTBM	0x	-	-	12.14	negative
113	NPC	mTBM	0x	-	-	13.56	negative
114	NPC	MKM	10x	-	-	17.08	negative
115	NPC	mTBM	10x	-	-	12.64	negative
116	NPC	MKM	10x	-	-	16.86	negative
117	NPC	mTBM	10x	-	-	12.87	negative
118	NPC	MKM	10x	37.24	-	17.9	negative
119	NPC	MKM	10x	37.23	34.66	17.12	negative
120	NPC	MKM	10x	-	-	17.3	negative
121	PPC	MKM	100x	19.98	16.42	16.91	positive
122	PPC	mTBM	100x	14.38	12.26	12.41	positive
123	PPC	MKM	100x	20.58	16.73	17.28	positive
124	PPC	mTBM	100x	15.01	12.62	12.65	positive
125	PPC	MKM	100x	19.7	15.99	16.64	positive
126	PPC	MKM	1000x	19.17	15.56	16.33	positive
127	PPC	mTBM	1000x	14.44	11.88	12.1	positive
128	PPC	MKM	1000x	19.67	15.96	16.64	positive
129	PPC	mTBM	1000x	15.11	12.46	12.63	positive
130	PPC	mTBM	1000x	14.13	12.12	12.33	positive
131	PAC	PCR MKM	-	21.34	17.47	17.45	positive
132	PAC	PCR mTBM	-	15.9	13.61	13.39	positive
133	PAC	PCR MKM	-	21.3	17.65	17.34	positive
134	PAC	PCR mTBM	-	16.5	14.26	13.97	positive
135	Xhc	MKM	-	19.98	16.26	16.91	positive
136	Xhc	mTBM	-	15.24	13.02	13.3	positive
137	NTC	PCR MKM	-	-	-	29.01	negative
138	NTC	PCR mTBM	-	-	-	-	negative

Annex C. Cont.

Lab. H – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
1	0x	0	TNTC	0	0	0	0	0	0	TNTC	0	0	0	0	0
1	10x	0	TNTC	0	0	0	0	0	0	TNTC	0	0	0	0	0
1	100x	0	16	0	0	0	0	0	0	50	0	0	0	0	0
1	1000x	0	1	0	0	0	0	0	0	4	0	0	0	0	0
2	0x	0	TNTC	0	0	0	0	0	0	TNTC	0	0	0	0	0
2	10x	0	TNTC	0	0	0	0	0	0	TNTC	0	0	0	0	0
2	100x	0	20	0	0	0	0	0	0	60	0	0	0	0	0
2	1000x	0	1	0	0	0	0	0	0	4	0	0	0	0	0
3	0x	1	50	1	1	1	1	1	0	50	0	0	0	0	0
3	10x	0	3	0	0	0	0	0	0	2	0	0	0	0	0
3	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
4	10x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
4	100x	10	5	1	1	1	2	1	12	3	1	1	1	3	1
4	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
5	10x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
5	100x	16	4	1	1	1	4	1	10	10	1	1	1	5	1
5	1000x	1	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0x	6	60	1	1	1	6	1	20	60	1	1	1	7	1
6	10x	0	5	0	0	0	0	0	1	6	0	0	0	0	0
6	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Annex C Cont.

Lab. H – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
7	0x	4	40	1	1	1	8	1	1	20	1	1	1	9	1
7	10x	0	3	0	0	0	0	0	0	1	0	0	0	0	0
7	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
8	10x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
8	100x	2	0	1	1	1	10	1	7	1	1	1	1	11	1
8	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
9	10x	15	0	0	0	0	0	0	25	80	0	0	0	0	0
9	100x	1	0	1	1	1	12	1	2	8	1	1	1	13	1
9	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
10	10x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
10	100x	0	14	0	0	0	0	0	0	18	0	0	0	0	0
10	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0x	4	25	1	1	1	26	1	3	50	1	1	1	27	1
11	10x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0x	7	45	0	0	0	0	0	60	40	0	0	0	0	0
12	10x	1	5	1	1	1	14	1	7	5	1	1	1	15	1
12	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Annex C Cont.

Lab. H – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
13	0x	80	0	0	0	0	0	0	65	40	0	0	0	0	0
13	10x	8	0	1	1	1	16	1	7	5	1	1	1	17	1
13	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0x	60	8	0	0	0	0	0	95	52	0	0	0	0	0
14	10x	6	1	1	1	1	18	1	10	5	1	1	1	19	1
14	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0x	1	300	1	1	1	28	1	1	>300	1	1	1	29	1
15	10x	0	25	0	0	0	0	0	0	40	0	0	0	0	0
15	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	0x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	10x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	100x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterility check	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PPC	0x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
PPC	10x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
PPC	100x	20	5	1	1	1	20	1	20	10	2	2	2	21, 22	2
PPC	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NPC	0x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
NPC	10x	TNTC	TNTC	0	0	0	0	0	TNTC	TNTC	0	0	0	0	0
NPC	100x	0	20	0	0	0	0	0	0	60	0	0	0	0	0
NPC	1000x	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Annex C. Cont.

Lab. H – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
1	3	MD5A	0x	19.85	19.95	21.83	positive
2	4	MD5A	100x	18.47	17.96	20.21	positive
3	4	MKM	100x	21.35	21.56	21.86	positive
4	5	MD5A	100x	18.42	17.68	19.65	positive
5	5	MKM	100x	20.3	20.45	21.7	positive
6	6	MD5A	0x	19.44	19.46	21.35	positive
7	6	MKM	0x	21.25	22	21.73	positive
8	7	MD5A	0x	18.45	17.52	18.69	positive
9	7	MKM	0x	19.67	19.02	20.72	positive
10	8	MD5A	100x	18.39	17.78	19.78	positive
11	8	MKM	100x	21.22	21.67	21.91	positive
12	9	MD5A	100x	18.53	17.82	19.73	positive
13	9	MKM	100x	20.09	20.01	21.69	positive
14	12	MD5A	10x	19.7	19.72	21.45	positive
15	12	MKM	10x	20.5	21.15	21.86	positive
16	13	MD5A	10x	17.54	17.27	19.4	positive
17	13	MKM	10x	21.17	21.7	21.91	positive
18	14	MD5A	10x	17.42	16.71	18.54	positive
19	14	MKM	10x	20.22	20.1	21.61	positive
20	PPC	MD5A	100x	18.86	18.5	20.79	positive
21	PPC	MKM	100x	21.72	22.2	21.84	positive
22	PPC	MKM	100x	21.1	21.44	21.84	positive
23	NaOH			N/A	N/A	22.06	negative
24	RC			19.79	19.99	21.62	positive
25	RC			22.23	23.68	22.09	positive
26	11	MD5A	0x	18.92	17.45	17.67	positive
27	11	MKM	0x	18.02	16.27	15.43	positive
28	15	MD5A	0x	18.1	16.78	17.22	positive
29	15	MKM	0x	19.24	18.48	19.11	positive
NTC				N/A	N/A	21.68	negative

Annex C. Cont.

Lab. I – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
1	0x	0	744						TNTC	TNTC					
1	10x	0	102	8	0	0	-	-	0	324					
1	100x	0	11						0	46	8	0	0	-	-
1	1000x	0	2						0	3					
2	0x	0	2256						TNTC	TNTC					
2	10x	0	304						0	652					
2	100x	0	28	8	0	0	-	-	0	80	8	0	0	-	-
2	1000x	0	1						0	6					
3	0x	320	4						368	25					
3	10x	36	3	8	8	8	8	8	40	2	8	8	8	8	8
3	100x	3	0						6	0					
3	1000x	0	0						1	0					
4	0x	TNTC	TNTC						TNTC	TNTC					
4	10x	204	23	8	8	8	8	8	72	368					
4	100x	11	0						20	72	8	8	8	8	8
4	1000x	1	0						1	5					
5	0x	288	124						TNTC	TNTC					
5	10x	64	5	8	8	8	8	8	28	56	8	8	8	8	8
5	100x	4	0						8	5					
5	1000x	0	0						1	0					
6	0x	8	55	8	8	8	8	7	2	184	3	2	3	3	2
6	10x	1	3						0	16					
6	100x	0	0						0	0					

Lab. I – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
6	1000x	0	0						0	0					
7	0x	8	380	8	8	8	8	8	7	176	7	7	7	7	7
7	10x	2	19						2	21					
7	100x	0	0						0	0					
7	1000x	0	0						0	0					
8	0x	68	56	8	8	8	8	8	28	124	8	8	8	8	8
8	10x	8	6						4	14					
8	100x	0	0						0	3					
8	1000x	0	0						0	0					
9	0x	TNTC	TNTC						TNTC	TNTC					
9	10x	152	64	8	8	8	8	8	96	264					
9	100x	15	2						16	64	8	8	8	7	7
9	1000x	1	0						1	8					
10	0x	TNTC	TNTC						0	TNTC					
10	10x	0	400						0	472					
10	100x	0	58	8	0	0	-	-	0	140					
10	1000x	0	6						0	16	8	0	0	-	-
11	0x	152	168						40	188					
11	10x	8	11	8	8	8	8	8	8	14	8	8	8	6	6
11	100x	1	1						0	2					
11	1000x	0	0						0	0					
12	0x	TNTC	TNTC						TNTC	TNTC					
12	10x	8	53	8	8	8	8	8	6	46	6	6	6	6	6

Annex C Cont.

Lab. I – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
12	100x	0	5						0	3					
12	1000x	0	1						0	0					
13	0x	8	314	8	8	6	6	6	4	200	4	4	3	3	2
13	10x	1	18						0	22					
13	100x	0	3						0	1					
13	1000x	0	0						0	0					
14	0x	8	122	8	2	2	2	1	10	192	8	6	6	6	6
14	10x	0	14						2	20					
14	100x	0	0						0	3					
14	1000x	0	0						0	0					
15	0x	7	TNTC	7	7	7	7	7	TNTC	TNTC					
15	10x	0	296						0	192					
15	100x	0	28						0	42	8	0	0	-	-
15	1000x	0	0						0	12					
sterility check	0x	0	0						0	0					
sterility check	10x	0	0						0	0					
sterility check	100x	0	0						0	0					
sterility check	1000x	0	0						0	0					
PPC	0x	TNTC	TNTC						TNTC	TNTC					
PPC	10x	240	148						272	96					
PPC	100x	28	44	8	8	8	8	8	36	55	8	8	4	4	4
PPC	1000x	3	5						4	11					
NPC	0x	TNTC	TNTC						TNTC	TNTC					

Annex C Cont.

Lab. I – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
NPC	10x	0	334						0	680					
NPC	100x	0	45						0	100					
NPC	1000x	0	8						0	9					

Annex C. Cont.

Lab. I – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
1	3	MKM	10×	14.81	14.34	9.16	positive
2	3	MKM	10×	13.23	14.94	8.35	positive
3	3	MKM	10×	13.86	14.48	7.65	positive
4	3	MKM	10×	10.43	14.03	9.98	positive
5	3	MKM	10×	12.83	14.04	8.34	positive
6	3	MKM	10×	14.53	14.25	9.62	positive
7	3	MKM	10×	11.34	14.44	7.99	positive
8	3	MKM	10×	10.61	14.79	8.29	positive
9	3	MD5A	10×	14.83	14.27	11.90	positive
10	3	MD5A	10×	16.06	14.81	10.75	positive
11	3	MD5A	10×	11.15	15.01	11.71	positive
12	3	MD5A	10×	15.56	16.29	12.76	positive
13	3	MD5A	10×	13.25	13.21	9.36	positive
14	3	MD5A	10×	11.01	14.83	9.44	positive
15	3	MD5A	10×	11.99	13.78	8.88	positive
16	3	MD5A	10×	13.07	14.39	10.77	positive
17	4	MKM	10×	10.09	14.34	7.92	positive
18	4	MKM	10×	14.89	14.39	8.93	positive
19	4	MKM	10×	11.61	14.33	8.36	positive
20	4	MKM	10×	12.46	14.45	7.21	positive
21	4	MKM	10×	12.71	14.61	9.31	positive
22	4	MKM	10×	13.85	14.52	9.81	positive
23	4	MKM	10×	13.81	13.98	8.14	positive
24	4	MKM	10×	13.25	14.25	8.42	positive
25	4	MD5A	100×	12.79	14.40	9.29	positive
26	4	MD5A	100×	12.20	14.19	9.86	positive
27	4	MD5A	100×	11.78	14.04	9.99	positive
28	4	MD5A	100×	12.07	14.18	8.82	positive
29	4	MD5A	100×	14.15	14.05	10.91	positive
30	4	MD5A	100×	13.19	14.29	10.50	positive
31	4	MD5A	100×	12.22	13.99	10.24	positive
32	4	MD5A	100×	14.38	13.99	10.58	positive
33	5	MKM	10×	16.09	16.11	9.43	positive
34	5	MKM	10×	14.49	13.76	9.95	positive
35	5	MKM	10×	12.23	15.19	8.26	positive
36	5	MKM	10×	14.70	14.61	7.30	positive
37	5	MKM	10×	13.87	13.59	8.85	positive
38	5	MKM	10×	14.43	13.55	12.31	positive
39	5	MKM	10×	13.43	14.30	11.43	positive
40	5	MKM	10×	14.33	13.69	9.84	positive
41	5	MD5A	10×	14.23	13.76	10.62	positive
42	5	MD5A	10×	13.27	14.27	10.56	positive
43	5	MD5A	10×	12.03	13.07	8.89	positive
44	5	MD5A	10×	14.15	14.17	11.93	positive
45	5	MD5A	10×	14.51	14.46	11.70	positive
46	5	MD5A	10×	13.23	14.93	12.24	positive

Annex C Cont.

Lab. I – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
47	5	MD5A	10×	14.99	14.63	10.76	positive
48	5	MD5A	10×	13.84	14.52	11.97	positive
49	6	MKM	1×	13.95	13.95	13.03	positive
50	6	MKM	1×	10.59	14.28	8.21	positive
51	6	MKM	1×	11.58	14.20	12.36	positive
52	6	MKM	1×	10.17	14.19	8.21	positive
53	6	MKM	1×	11.24	14.75	7.25	positive
54	6	MKM	1×	14.84	14.48	8.23	positive
55	6	MKM	1×	Undetermined	Undetermined	8.63	negative
56	6	MKM	1×	13.91	13.15	12.02	positive
57	6	MD5A	1×	Undetermined	Undetermined	12.81	negative
58	6	MD5A	1×	14.41	12.81	9.50	positive
59	6	MD5A	1×	13.32	13.38	10.16	positive
60	7	MKM	1×	12.39	14.19	9.16	positive
61	7	MKM	1×	12.11	13.69	12.48	positive
62	7	MKM	1×	10.89	13.28	9.38	positive
63	7	MKM	1×	10.05	13.83	7.28	positive
64	7	MKM	1×	10.45	13.84	8.21	positive
65	7	MKM	1×	13.41	15.99	7.86	positive
66	7	MKM	1×	11.34	14.53	7.36	positive
67	7	MKM	1×	9.57	14.25	9.11	positive
68	7	MD5A	1×	14.88	14.10	11.49	positive
69	7	MD5A	1×	10.97	14.29	9.60	positive
70	7	MD5A	1×	15.46	14.98	9.28	positive
71	7	MD5A	1×	10.24	14.54	9.77	positive
72	7	MD5A	1×	11.65	13.89	9.80	positive
73	7	MD5A	1×	11.87	13.78	10.67	positive
74	7	MD5A	1×	12.32	14.36	8.35	positive
75	8	MKM	1×	14.85	13.69	12.52	positive
76	8	MKM	1×	9.76	14.07	8.15	positive
77	8	MKM	1×	12.69	14.18	7.57	positive
78	8	MKM	1×	11.17	14.77	7.64	positive
79	8	MKM	1×	13.82	13.97	12.69	positive
80	8	MKM	1×	14.69	14.47	7.75	positive
81	8	MKM	1×	12.62	14.91	8.36	positive
82	8	MKM	1×	11.90	14.67	8.60	positive
83	8	MD5A	1×	14.03	13.55	9.11	positive
84	8	MD5A	1×	12.64	15.15	8.82	positive
85	8	MD5A	1×	13.45	13.84	8.98	positive
86	8	MD5A	1×	13.02	13.95	10.22	positive
87	8	MD5A	1×	11.79	13.96	9.77	positive
88	8	MD5A	1×	11.44	15.07	9.45	positive
89	8	MD5A	1×	14.33	14.58	12.02	positive
90	8	MD5A	1×	14.06	14.14	9.83	positive
91	9	MKM	10×	15.35	14.73	12.38	positive
92	9	MKM	10×	16.34	14.86	12.44	positive

Annex C Cont.

Lab. I – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
93	9	MKM	10×	14.69	14.83	12.40	positive
94	9	MKM	10×	14.88	14.83	11.26	positive
95	9	MKM	10×	10.96	14.54	10.16	positive
96	9	MKM	10×	14.67	14.90	11.84	positive
97	9	MKM	10×	11.25	14.00	10.21	positive
98	9	MKM	10×	14.74	13.88	10.76	positive
99	9	MD5A	100×	11.70	14.07	8.70	positive
100	9	MD5A	100×	12.53	13.60	9.24	positive
101	9	MD5A	100×	13.77	14.42	11.43	positive
102	9	MD5A	100×	14.76	14.38	10.06	positive
103	9	MD5A	100×	15.31	14.39	11.63	positive
104	9	MD5A	100×	16.07	14.62	11.74	positive
105	9	MD5A	100×	12.48	14.51	11.46	positive
106	9	MD5A	100×	Undetermined	Undetermined	32.25	invalid
107	11	MKM	10×	9.61	12.03	9.70	positive
108	11	MKM	10×	9.83	13.94	10.14	positive
109	11	MKM	10×	11.50	13.51	9.56	positive
110	11	MKM	10×	10.49	13.72	9.77	positive
111	11	MKM	10×	12.01	13.41	9.98	positive
112	11	MKM	10×	15.54	14.68	12.28	positive
113	11	MKM	10×	12.67	14.32	10.87	positive
114	11	MKM	10×	9.36	12.14	8.75	positive
115	11	MD5A	10×	13.19	13.45	12.87	positive
116	11	MD5A	10×	13.95	14.22	11.43	positive
117	11	MD5A	10×	11.74	12.80	9.09	positive
118	11	MD5A	10×	11.69	13.41	9.48	positive
119	11	MD5A	10×	12.08	14.02	9.98	positive
120	11	MD5A	10×	14.18	14.08	9.83	positive
121	12	MKM	10×	14.27	14.62	11.60	positive
122	12	MKM	10×	13.85	13.57	11.60	positive
123	12	MKM	10×	15.75	14.62	12.37	positive
124	12	MKM	10×	12.08	14.86	11.32	positive
125	12	MKM	10×	15.45	14.40	11.10	positive
126	12	MKM	10×	15.33	14.93	11.87	positive
127	12	MKM	10×	14.98	14.04	11.99	positive
128	12	MKM	10×	15.93	14.62	12.01	positive
129	12	MD5A	10×	11.55	14.04	12.88	positive
130	12	MD5A	10×	12.34	14.35	9.08	positive
131	12	MD5A	10×	11.94	14.33	10.87	positive
132	12	MD5A	10×	12.49	14.29	9.84	positive
133	12	MD5A	10×	10.58	13.77	9.48	positive
134	12	MD5A	10×	15.21	15.98	9.33	positive
135	13	MKM	1×	10.06	15.74	11.93	positive
136	13	MKM	1×	13.90	14.09	11.92	positive
137	13	MKM	1×	9.51	15.05	8.92	positive
138	13	MKM	1×	10.85	14.25	9.79	positive

Annex C Cont.

Lab. I – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
139	13	MKM	1×	14.33	13.82	11.04	positive
140	13	MKM	1×	13.85	13.69	11.98	positive
143	13	MD5A	1×	11.61	14.99	10.85	positive
144	13	MD5A	1×	27.49	Undermined	15.22	positive
145	13	MD5A	1×	Undermined	Undermined	15.99	negative
146	14	MKM	1×	18.60	18.94	16.53	positive
147	14	MKM	1×	Undermined	Undermined	14.99	negative
148	14	MD5A	1×	9.98	14.49	8.64	positive
149	14	MD5A	1×	14.73	14.40	9.77	positive
150	14	MD5A	1×	14.39	14.61	10.98	positive
151	14	MD5A	1×	15.26	14.47	9.61	positive
152	14	MD5A	1×	14.54	14.08	11.64	positive
153	14	MD5A	1×	16.02	16.07	10.95	positive
154	15	MKM	1×	12.47	13.69	13.42	positive
155	15	MKM	1×	11.88	13.78	9.55	positive
156	15	MKM	1×	9.44	14.95	8.93	positive
157	15	MKM	1×	14.49	13.67	10.24	positive
158	15	MKM	1×	13.59	14.05	11.51	positive
159	15	MKM	1×	12.37	14.95	8.90	positive
160	15	MKM	1×	11.37	13.32	9.93	positive
161	PPC	MKM	100×	15.53	15.44	12.18	positive
162	PPC	MKM	100×	14.10	15.63	10.61	positive
163	PPC	MKM	100×	14.29	13.83	10.77	positive
164	PPC	MKM	100×	15.70	14.81	12.32	positive
165	PPC	MKM	100×	15.64	15.43	13.13	positive
166	PPC	MKM	100×	15.71	14.72	11.53	positive
167	PPC	MKM	100×	15.47	14.61	12.17	positive
168	PPC	MKM	100×	15.77	14.51	12.78	positive
169	PPC	MD5A	100×	16.28	15.71	13.19	positive
170	PPC	MD5A	100×	13.22	15.56	10.09	positive
171	PPC	MD5A	100×	14.70	13.52	11.40	positive
172	PPC	MD5A	100×	14.76	14.95	10.47	positive
PPC				18.37	16.68	13.64	positive
NPC				Undermined	Undermined	17.51	negative
PAC-Xhc-1				17.89	18.69	15.80	positive
PAC-Xhx-2				20.95	19.21	16.49	positive
NTC				Undermined	Undermined	32.02	

Annex C. Cont.

Lab. J – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
1	0x														
1	10x	5	120	3	3	3	1.2, 1.3, 1.4	0	6	150	3	2	3	1.5, 1.6, 1.7	0
1	100x	1	24	1	1	1	1.1	0							
1	1000x														
2	0x	1	>250	1	1	1	2.1	0	30	>250	3	2	3	2.4, 2.5, 2.6	0
2	10x								2	150	2	2	2	2.2, 2.3	0
2	100x														
2	1000x														
3	0x	7	3	3	3	3	3.1, 3.2, 3.3	3	14	31	3	3	3	3.4, 3.5, 3.6	3
3	10x														
3	100x														
3	1000x														
4	0x														
4	10x	161	0	3	3	3	4.1, 4.2, 4.3	3	218	>200	3	3	3	4.4, 4.5, 4.6	3
4	100x														
4	1000x														
5	0x														
5	10x	144	0	3	3	3	5.1, 5.2, 5.3	3	188	50	3	3	3	5.4, 5.5, 5.6	3
5	100x														
5	1000x														

Annex C Cont.

Lab. J – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
6	0x	0	6	0					10	32	6	5	6	6.1, 6.2, 6.3, 6.4, 6.5, 6.6	5
6	10x														
6	100x														
6	1000x														
7	0x	11	8	3	3	3	7.1, 7.2, 7.3	3	13	26	3	3	3	7.4, 7.5, 7.6	3
7	10x														
7	100x														
7	1000x														
8	0x	13	11	3	3	3	8.1, 8.2, 8.3	3	17	>200	3	2	3	8.4, 8.5, 8.6	2
8	10x														
8	100x														
8	1000x														
9	0x														
9	10x	129	2	3	3	3	9.1, 9.2, 9.3	3	147	180	3	3	3	9.4, 9.5, 9.6	3
9	100x														
9	1000x														
10	0x	0	>250	0											
10	10x	0	80	0					3	150	3	3	3	10.1, 10.2, 10.3	0
10	100x														
10	1000x														

Lab. J – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
11	0x	7	7	3	3	3	11.1, 11.2, 11.3	3	8	>200	4	3	4	11.4, 11.5, 11.6, 11.7	3
11	10x														
11	100x														
11	1000x														
12	0x	25	10	3	3	3	12.1, 12.2, 12.3	3	40	>500	3	3	3	12.4, 12.5, 12.6	3
12	10x								1	110					
12	100x														
12	1000x														
13	0x	7	11	3	3	3	13.1, 13.2, 13.3	3	12	24	3	3	3	13.4, 13.5, 13.6	3
13	10x														
13	100x														
13	1000x														
14	0x	41	8	3	3	3	14.1, 14.2, 14.3	3	34	40	3	3	3	14.4, 14.5, 14.6	3
14	10x														
14	100x														
14	1000x														
15	0x	181	7	3	3	3	15.4, 15.5, 15.6	3	327	30	3	3	3	15.1, 15.2, 15.3	3
15	10x														
15	100x														
15	1000x														

Annex C Cont.

Lab. J – Table 1		MKM medium							MD5A medium						
Sample	Dilution	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive	Suspect Xhc colonies	Other colonies	Colonies transferred to YDC	Colonies suspect on YDC	Colonies tested with PCR	Specified colonies on Datasheet 5	Colonies PCR positive
sterility check	0x	0	0	0					0	0	0				
sterility check	10x														
sterility check	100x	0	35*	0											
sterility check	1000x														
PPC	0x														
PPC	10x	131	2	3	3	3	17.4, 17.5, 17.6	3	191	58	3	3	3	17.1, 17.2, 17.3	3
PPC	100x														
PPC	1000x														
NPC	0x	2	>300	2	2	2	16.1, 16.2	0							
NPC	10x								0	90	0				
NPC	100x														
NPC	1000x														

*) On one of the two E2 dilution plates (MKM), other colonies were observed. While both E0 dilution plates were empty. Most probably, a contamination was present on one of the two MKM plates.

Annex C. Cont.

Lab. J – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
1.1	1	MKM	100x	>40	>40	16.96	negative
1.2	1	MKM	10x	>40	>40	16.01	negative
1.3	1	MKM	10x	>40	>40	16.05	negative
1.4	1	MKM	10x	>40	>40	16.47	negative
1.5	1	MD5A	10x	>40	>40	15.21	negative
1.7	1	MD5A	10x	>40	>40	15.47	negative
2.1	2	MKM	0x	>40	>40	15.64	negative
2.2	2	MD5A	10x	>40	>40	19.54	negative
2.3	2	MD5A	10x	>40	>40	20.30	negative
2.4	2	MD5A	0x	>40	>40	20.64	negative
2.5	2	MD5A	0x	>40	>40	17.69	negative
3.1	3	MKM	0x	15.79	14.91	14.37	positive
3.2	3	MKM	0x	16.25	15.26	15.16	positive
3.3	3	MKM	0x	15.28	14.41	14.28	positive
3.4	3	MD5A	0x	15.35	14.25	14.23	positive
3.5	3	MD5A	0x	13.86	13.06	13.63	positive
3.6	3	MD5A	0x	14.58	13.56	13.59	positive
4.1	4	MKM	10x	15.65	14.83	15.01	positive
4.2	4	MKM	10x	15.05	14.27	14.26	positive
4.3	4	MKM	10x	15.61	14.79	14.79	positive
4.4	4	MD5A	10x	15.53	14.80	14.57	positive
4.5	4	MD5A	10x	16.22	15.70	15.21	positive
4.6	4	MD5A	10x	15.16	14.41	14.41	positive
5.1	5	MKM	10x	15.48	14.63	14.51	positive
5.2	5	MKM	10x	15.62	14.55	14.71	positive
5.3	5	MKM	10x	14.88	14.03	14.20	positive
5.4	5	MD5A	10x	14.90	14.00	14.28	positive
5.5	5	MD5A	10x	13.77	13.05	12.92	positive
5.6	5	MD5A	10x	15.28	14.43	14.43	positive
6.2	6	MD5A	0x	14.94	13.99	13.80	positive
6.3	6	MD5A	0x	12.99	12.33	14.24	positive
6.4	6	MD5A	0x	14.84	13.99	14.45	positive
6.5	6	MD5A	0x	16.15	15.48	14.85	positive
6.6	6	MD5A	0x	13.17	12.50	14.53	positive
7.1	7	MKM	0x	12.86	12.44	23.45	positive
7.2	7	MKM	0x	12.83	12.14	20.44	positive
7.3	7	MKM	0x	13.33	12.96	21.10	positive
7.4	7	MD5A	0x	13.68	13.32	22.02	positive
7.5	7	MD5A	0x	15.68	14.89	14.68	positive
7.6	7	MD5A	0x	14.44	13.52	13.61	positive
8.1	8	MKM	0x	15.16	14.39	14.53	positive
8.2	8	MKM	0x	15.07	14.31	14.27	positive
8.3	8	MKM	0x	15.03	14.48	14.75	positive
8.5	8	MD5A	0x	15.43	15.15	15.59	positive
8.6	8	MD5A	0x	15.60	14.91	14.82	positive
9.1	9	MD5A	10x	14.72	14.13	14.04	positive

Annex C Cont.

Lab. J – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
9.2	9	MD5A	10x	17.13	16.39	16.55	positive
9.3	9	MD5A	10x	14.32	13.49	13.55	positive
9.4	9	MKM	10x	13.22	12.46	13.59	positive
9.5	9	MKM	10x	15.85	15.38	14.79	positive
9.6	9	MKM	10x	15.17	14.61	14.42	positive
10.1	10	MD5A	10x	>40	>40	14.47	negative
10.2	10	MD5A	10x	>40	>40	18.72	negative
10.3	10	MD5A	10x	>40	>40	19.21	negative
11.1	11	MKM	0x	14.38	13.89	13.61	positive
11.2	11	MKM	0x	15.39	14.79	14.53	positive
11.3	11	MKM	0x	15.38	14.70	14.67	positive
11.4	11	MD5A	0x	15.02	14.29	14.00	positive
11.5	11	MD5A	0x	15.09	14.62	14.27	positive
11.6	11	MD5A	0x	15.02	14.43	14.40	positive
12.1	12	MKM	0x	15.41	14.90	14.55	positive
12.2	12	MKM	0x	15.61	15.16	14.93	positive
12.3	12	MKM	0x	15.59	14.88	14.73	positive
12.4	12	MD5A	0x	25.32	25.59	14.65	positive
12.5	12	MD5A	0x	29.54	29.65	14.91	positive
12.6	12	MD5A	0x	15.29	14.66	14.48	positive
13.1	13	MKM	0x	16.07	15.68	16.53	positive
13.2	13	MKM	0x	15.67	15.36	15.52	positive
13.3	13	MKM	0x	15.59	15.20	15.25	positive
13.4	13	MD5A	0x	13.22	12.56	13.18	positive
13.5	13	MD5A	0x	14.29	13.74	13.56	positive
13.6	13	MD5A	0x	14.43	13.69	13.92	positive
14.1	14	MKM	0x	14.06	13.44	13.49	positive
14.2	14	MKM	0x	15.38	14.79	14.69	positive
14.3	14	MKM	0x	15.33	14.74	14.53	positive
14.4	14	MD5A	0x	15.04	14.63	14.55	positive
14.5	14	MD5A	0x	14.70	14.22	14.19	positive
14.6	14	MD5A	0x	14.86	14.31	13.93	positive
15.1	15	MD5A	0x	15.53	15.21	14.71	positive
15.2	15	MD5A	0x	15.22	14.72	14.14	positive
15.3	15	MD5A	0x	14.34	13.89	13.60	positive
15.4	15	MKM	0x	15.82	15.41	15.22	positive
15.5	15	MKM	0x	14.58	13.87	14.07	positive
15.6	15	MKM	0x	15.54	15.12	14.74	positive
16.1	NPC	MKM	0x	>40	>40	26.75	negative
16.2	NPC	MKM	0x	>40	>40	15.53	negative
17.1	PPC	MD5A	10x	14.64	14.03	13.84	positive
17.2	PPC	MD5A	10x	15.84	15.40	15.50	positive
17.3	PPC	MD5A	10x	15.38	14.70	14.59	positive
17.4	PPC	MKM	10x	13.98	13.60	14.23	positive
17.5	PPC	MKM	10x	13.67	13.27	14.27	positive
17.6	PPC	MKM	10x	13.82	13.20	13.75	positive

Annex C Cont.

Lab. J – Table 2 Colony	Sample	Medium	Dilution	Cq MVS-Xhc	Cq Temple-Xhc	Cq Wu	Result
1.6, non-suspect	1	MD5A	10x	>40	>40	14.15	negative
2.6, non-suspect	2	MD5A	0x	>40	>40	14.18	negative
6.1, non-suspect	6	MD5A	0x	>40	>40	14.95	negative
8.4, non-suspect	8	MD5A	0x	>40	>40	13.22	negative
11.7, non-suspect	11	MD5A	0x	>40	>40	14.88	negative
Xhc strain 301	x	x	x	15.82	14.96	14.54	positive
Xhc strain 306	x	x	x	14.26	13.24	13.65	positive
PAC-Xhc-1	x	x	x	16.46	15.60	15.12	positive
PAC-Xhx-2	x	x	x	21.47	21.04	19.99	positive
NTC	x	x	x	>40	>40	32.12	negative