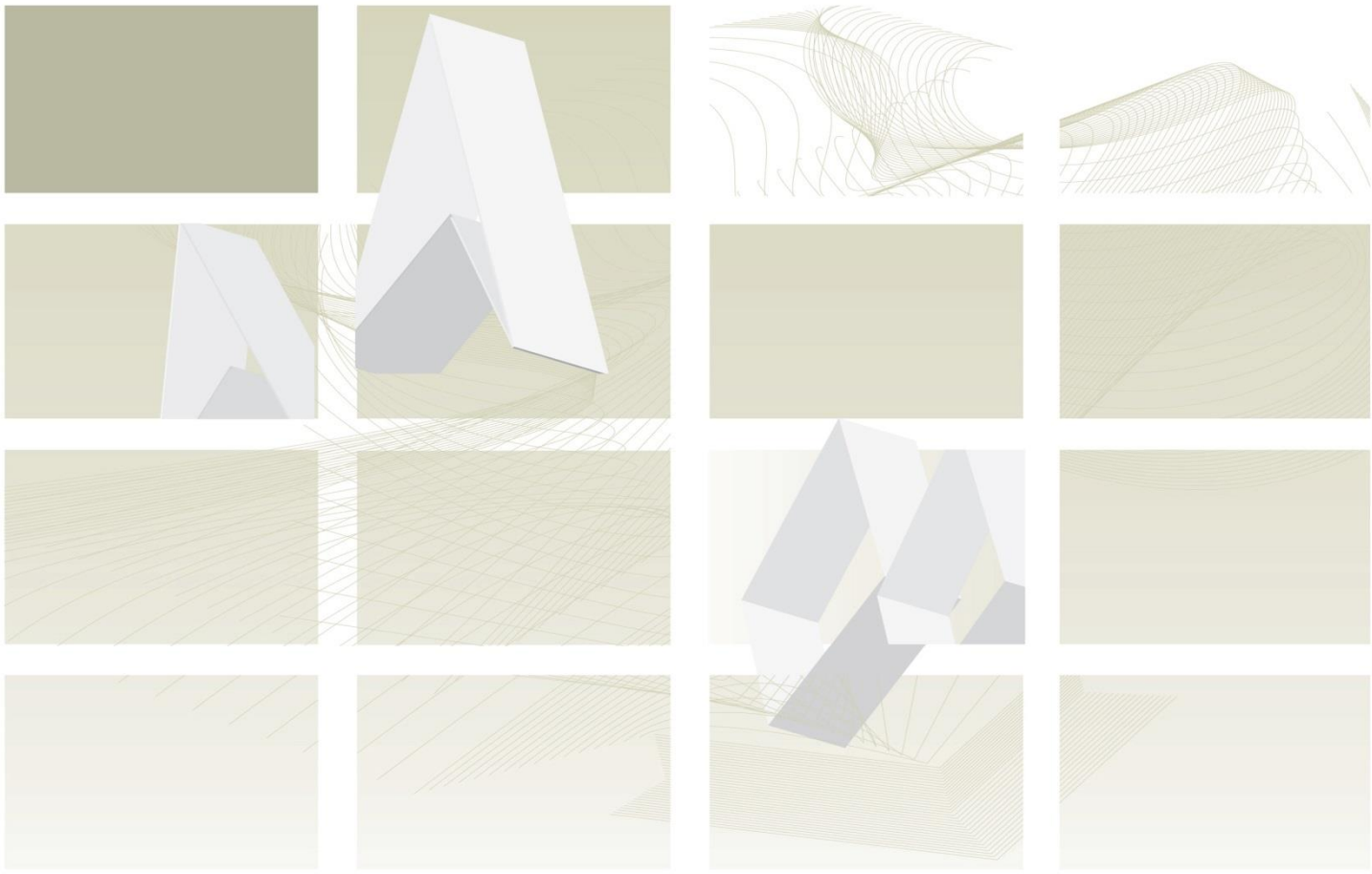
Public Health England

NHS

UK Standards for Microbiology Investigations

Review of users' comments received by
Working group for microbiology standards in clinical virology/serology

Q 4 Good practice when performing molecular amplification assays



NICE accredited
www.nice.org.uk/accreditation

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

First consultation: 11/08/2017 – 25/08/2017

Version of document consulted on: Q 4dq+

Proposal for changes

Comment number	1		
Date received	14/08/2017	**Lab name**	PHE Porton
Section	Multiple		
Comment			
Please alter the title (and text throughout) of this document to remove the term good laboratory practice as this has legal meaning in a quality context. Perhaps rename it as good scientific practice? Use of the term GLP will attract an Audit to the standards of GLG from the MHRA.			
Evidence			
MHRA website http://www.legislation.gov.uk/uksi/1999/3106/regulation/3/made			
Financial barriers			
Not completed.			
Health benefits			
Failure to address this issue may result in an audit failure by MHRA which would affect the reputation of PHE.			
Recommended action	**NONE** This issue has already been addressed in the document prior to consultation. The version that went to consultation had the updated title “Good practice when performing molecular amplification assays” and amendments made throughout the document. The amendment table on page 4 has the updated information.		

Comment number	2		
Date received	15/08/2017	**Professional body**	ACM ,UKCVN & UKAS assessor
Section	6 Quality issues		
Comment			
Although I accept that the document states 'it is important to demonstrate that assays are performing consistently....'I am surprised that there is no mention of demonstration of spectral calibration in real time PCR or evidence that amplification platforms are meeting the required temperature calibration as required by ISO 15189 -both being critical for the			

procedures to obtain reliable results. The use of external biological controls can be used as a surrogate as long as the data is collected and analysed appropriately.	
Evidence	
Not completed.	
Financial barriers	
Some small expense.	
Health benefits	
No.	
Recommended action	**ACCEPT** This comment will be considered in the UK SMI Q1 document: Evaluations, Validations and Verifications of Diagnostic Tests when it comes up for review. A change request has been raised to that effect.

Comment number	3		
Date received	22/08/2017	**Lab name**	National Infection Service, Public Health England
Section	Multiple		
Comment			

a. Amend throughout all the hpa.org.uk website addresses to reflect the 01/04/2014 change from HPA to PHE. The links are correctly redirected as a legacy function, but need updating to their new final destination URLs in the reviewed document.

Several paragraphs to be added to the document:

b. Add para at the end of Introduction: Next-Generation Sequencing (NGS) is emerging as a powerful new diagnostic technique. Almost all applications involve one or more PCR amplification steps, so the mitigating practices described herein also apply to NGS applications. Additional contamination risks are presented by NGS, particularly surrounding the use of adaptor/index molecules throughout an NGS workflow. Further steps to control these risks will be essential, but are beyond the scope of this document.

c. Organisation of Work, para 6: When discussing UV decontamination, it is imperative to state that weekly monitoring of bulb strength is necessary to ensure sufficient decontamination effect

d. Add para at the end of 1.1 Organisation of Work: It is very important that the area where specimens are received into the testing facility remains PCR 'clean', with no cloned or PCR-amplified material being handled. If such material is received by the testing laboratory, a separate, dedicated area for processing should be available, with its own equipment, lab coats, etc.

e. Title of 2.1: change to Physical and Temporal Separation of Pre-PCR and Post-PCR Assay Stages

f. Add para at the end of 2.1: Where multiple overlapping PCR-based assays are being performed, in order to minimise the possibility of adventitious transfer of downstream material into clean areas, it is advantageous to perform clean tasks early in the working day, and 'dirty' tasks later, once the clean tasks have been completed, in order to minimise the possibility of adventitious transfer of downstream material into clean areas.

g. 2.2, para 2: Change practise (verb) to practice (noun)

h. Amend bullet point 1, section 4 Selection of Controls: A positive amplification control: this should normally be an extract that amplifies weakly but consistently within an acceptable range. A decline in assay performance may not be detected when using a high copy-number positive as this may still give a signal. Use of a strong positive is also an unnecessary risk as it can be a possible reservoir of contamination.

Evidence

Not completed.

Financial barriers

No.

Health benefits

The entire scope of this and other SMI documents is to ensure accurate conduct of diagnostic pathology testing, in this case using nucleic acid amplification techniques. Consequently every component of the SMI has health benefits, side effects and risks that might affect the development of this UK SMI. I'm not sure this question is appropriate!

Recommended action	a. **NONE** This has already been addressed in the updated version of the document sent out on consultation. There is a possibility that the version of the document that was looked at is the version under review on the gov.uk website. b. **ACCEPT** Information on NGS has been added to the document accordingly. c. **ACCEPT** This has been updated in the document accordingly. d. **ACCEPT** This has been updated in the document accordingly. e. **NONE** It was agreed by the Working Group members that the title heading be kept as the same. f. **NONE**

	It was agreed by the Working Group members that the comment is not practical in laboratories and so this should not be mentioned in the document. g. **NONE** This has already been updated in the document accordingly. h. **ACCEPT** This has already been updated in the document accordingly.

Comment number	4		
Date received	22/08/2017	**Lab name**	PHE Public Health Laboratory Birmingham
Section	1.1; 3; 4		
Comment			
a. 1.1: I suggest changing the sentence Workbooks that have been in contaminated areas should not be taken into clean PCR areas. To workbooks or worksheets that have been in contaminated areas should not be taken into clean PCR areas. Worksheets are a much more likely item in a diagnostic lab than a workbook. b. 3: I suggest changing the sentence Mastermixes should be subjected to minimal thawing and put on ice as soon as possible to Mastermixes should be subjected to minimal thawing and put on ice or a cooling block as soon as possible. Not many busy diagnostic labs will still be using ice as cool blocks are so much cleaner and more convenient. c. 4: I suggest changing the sentence Demonstration of the internal control sequence by PCR in a duplexed reaction with the target ... to Demonstration of the internal control sequence by PCR in a multiplexed reaction with the target... In many labs, single target PCRs are not very common and the internal control will be part of a triplex or quadruplex assay.			
Evidence			
Experience.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	a. **NONE** This has already been updated using the word “documentation”. b. **NONE**		

	This has already been updated in the document. c. **ACCEPT** This has been updated in the document accordingly.

Comment number	5		
Date received	24/08/2017	**Lab name**	Microbiology Dept, Belfast Trust
Section	Multiple sections		
Comment			
I would like to make a few comments on the GLP when performing molecular amplification assays SMI. While this is a very informative and useful document I think the layout overall is not fluid- there are multiple sections where the order seems mixed up. This document details GLP for molecular assays with a traditional setup- there is no reference to newer molecular working set ups where total enclosed automated platforms are utilised in a unidirectional way from specimen processing, extraction to PCR set up.			
Evidence			
a. Section 1.1: This is a bit confused- needs reorganised. i. Organisation of work: 'Avoid entering pre-amplification rooms immediately after working in rooms where products, cloned material etc are handled'- This is unrealistic- how does one define immediately? ii. Also there are specific precautions in place as detailed in section 2.6 to prevent/mitigate the risks of contamination. iii. 'All new members .... must be trained in use of PCR facilities'- this could be more specific iv. 'For reverse transcription specific precautions are necessary...' What are these specific precautions? b. Section 2.1: It is essential to have some reference in section 2.1 to automated start to finish systems - these are becoming much more commonplace in molecular UK laboratories and the SMI needs to address the GLP when using these systems. c. Section 2.2: 'The unidirectional Workflow'- may be better to include this in '1.1 Organisation of Work' section. d. Section 2.3: 'the PCR machine room' should be changed to PCR amplification room e. Section 2.4: 'The nucleic Acid extraction room'... This should be changed to 'Extraction and PCR setup room.... many UK labs no longer have separate rooms for these- labs using start to finish automated high throughput systems (sample processing, extraction and PCR setup) must be in the same room...			

f. Section 2.5: 'Individual users' PCR programs in the thermalcyclers should not be edited.....' this should be deleted as this is a policy that should be decided at local individual level.

g. Section 3: 'Mastermixes should be subjected to minimal thawing and put on ice....' This should be changed to ' Mastermixes should be subjected to minimal thawing and handled as per manufacturer instructions'...

h. Section 4: Selection of controls A positive amplification control...within an acceptable range' should be changed to 'within a locally defined range'

i. Section 4: Selection of controls:

   i. Point 4 - 'Extraction controls' This may need rephrased- seems a bit confused- are we really talking about process controls??

   ii. Point 5: Important to include that a control of the whole process using a separate PCR reaction is acceptable.

j. Section 5: 'Regular environmental swabbing is recommended..' should be changed to 'Environmental swabbing can be useful' Environmental sampling can certainly be useful but recommending it as a regular necessity in a molecular laboratory can result in difficulties for individual laboratories- Do you test for every PCR target? How and where to swab? How often? What to do if positive? Is there a document that refers to best practice for this?

k. References:

   Reference 7: Is it appropriate to reference a company in this SMI?

Financial barriers

No.

Health benefits

No.

Recommended action	a. i. **ACCEPT** This has been addressed in the document accordingly. ii. **NONE** This has already been addressed in the document accordingly. iii. **NONE** This has already been addressed in the document accordingly. iv. **NONE** This has already been addressed in the document accordingly. b. **ACCEPT** This has been updated in the document. c. **NONE**

	This has already been addressed in the document accordingly and will not be moved into the section 1.1 in the document. d. **ACCEPT** This has been updated in the document accordingly. e. **ACCEPT** This has been mentioned in the document where appropriate. f. **NONE** It is best practice to have only a limited capability for editing programs and all amendments will need to be revalidated and so this will remain in the document as it is useful for the users to know. g. **ACCEPT** This has been updated in the document accordingly. h. **ACCEPT** This has been updated in the document accordingly. i. i. **ACCEPT** This has been updated in the document accordingly. ii. **NONE** The information will remain as it is in the document. j. **ACCEPT** This has been updated in the document. k. **NONE** This has already been removed from the document.

Comment number	6		
Date received	24/08/2017	**Professional body**	Society for Applied Microbiology
Section	a. Introduction b. 1.1 Organisation of work c. 2 Specimen Processing d. 5 Other considerations to avoid contamination e. 6 Quality Assurance		
Comment			
a. i. [Paragraph 2] In addition to clones DNA and virus cell cultures, microbes within the environment are a significant source of contamination.			

ii. [Paragraph 4] Another significant, or possibly greater, risk is through cross-contamination of different reactions prepared at the same time. Also, the contamination of master stocks (eg oligonucleotide stocks) by DNA templates is a major threat to be considered.

b.

i. [Paragraph 2] The setup of a formal induction process should be a must, rather than a recommendation, to ensure all workers have a standardised introduction to a particular laboratory, regardless of prior experience.

ii. [Paragraph 3] We would recommend that batch numbers be recorded in a centralised manner for the laboratory, to improve traceability.

iii. [Paragraph 4] Some key examples of when gloves ought to be changed would be beneficial, for example in between the processing of individual patient samples.

iv. [Paragraph 5] Many laboratories are not able to arrange PCR work across separate rooms and therefore must rely on segregation of areas to separate pre- and post-PCR work. This means that changing laboratory coats between work areas is therefore not practical.

v. [Paragraph 7] Should also be clear here that when a fresh reagent arrives, it should be aliquoted into the amounts required for single use straight away. This is also relevant to section 2.3.

vi. [Paragraph 8] We would recommend that benches be wiped with disinfection solution before and after each procedure as necessary.

c.

i. 2.2 The unidirectional workflow: [Paragraph 3] It is also worth considering how air pressure and flow in these rooms/areas can be adjusted to minimise contamination risk. For example, the amplification room should be under negative air pressure to prevent PCR products potentially escaping and contaminating other rooms/areas of the laboratory. 2.3

ii. Reagent preparation clean room: [Paragraph 2] It may be necessary to clean the workspace when changing between different primers and other reagents.

iii. 2.4. The nucleic acid extraction room [Paragraph 2] 'cDNA' should not be used here, as it may be misinterpreted as complementary DNA, which is not referred to in this section.

d.

i. [Paragraph 1] For Real Time PCR, these glycosylases can show both advantages and limitations which ought to be recognised. Total elimination of contaminants is not always accomplished using this technique, particularly where PCR product length is short. Also inclusion of UDGs may reduce amplification efficiency and thereby delay or prevent detection, when only one or a few target molecules are present. Heat-labile forms of the enzyme are available to minimise residual UDG activity after PCR.

ii. [Paragraph 2] For consistency, the concentration of sodium hypochlorite should be stated.

iii. [Paragraph 3] It may be worth explicitly highlighting the risk of pipette contamination through not using aerosol-barrier tips. In addition, although

perhaps obvious, it could be specified that DNAse- and RNAse-free pipette tips, which have been gamma irradiated, should be utilised. e. [Paragraph 1] We would advise that 'deep clean' decontamination procedures be put in place and employed in the event that a major lab disruption takes place (for instance during emergency evacuations or the entrance of building contractors into the PCR suite).	
Evidence	
Not completed.	
Financial barriers	
Not completed.	
Health benefits	
Not completed.	
Recommended action	a. i. **ACCEPT** This has been updated in the document. ii. **ACCEPT** This has been updated in the document. b. i. **ACCEPT** This has been updated in the document. ii. **ACCEPT** This has been updated in the document. iii. **ACCEPT** This has been updated in the document. iv. **NONE** This will remain as described in the document. It may be necessary to review as part of a local risk assessment for the process. v. **ACCEPT** This has been updated in the document. vi. **ACCEPT** This has been updated in the document. c. i. **NONE** This is outside the remit of the UK SMIs. ii. **ACCEPT** This has been updated in the document. iii. **ACCEPT** This has been removed from the document. d. i. **ACCEPT**

	This has been updated in the document accordingly. ii. **ACCEPT** This will be updated in the document. iii. **ACCEPT** This has been updated in the document accordingly. e. **ACCEPT** This has been updated in the document accordingly.

Comment number	7		
Date received	25/08/2017	**Professional body**	Royal Cornwall Hospitals Trust
Section	a. 5 b. 6		
Comment			
a. Section 5, 2nd bullet point - it would be useful to know the concentration of sodium hypochlorite required. Also is it better to state HCl as hydrochloric acid? b. Section 6, 3rd paragraph - 'characterized' (English or American spelling?)There is no mention of commercial reagents such as 'DNA away' for decontamination.			
Evidence			
Not completed.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	a. **ACCEPT** This will be updated in the document. b. **NONE** This has already been updated in the document.		

Second consultation: 05/09/2017 – 19/09/2017

Version of document consulted on: Q 4dw+

Proposal for changes

Comment number	1	

Date received	05/09/2017	**Lab name**	Sheffield Teaching Hospital NHS Trust
Section	Multiple sections		
Comment			
Not all molecular assays are PCR. Either specify the UK SMI as PCR assays good laboratory practice or stop using PCR and replace with molecular amplification assays eg SDA, TMA etc.			
Evidence			
Not completed.			
Financial barriers			
Not completed.			
Health benefits			
Not completed.			
Recommended action	**NONE** The title of the UK SMI will remain as it is and other molecular amplification assays have been included in the introduction.		

Comment number	2		
Date received	05/09/2017	**Lab name**	PHE NIS Porton
Section	Several		
Comment			
Minor typo and three suggested additions - see tracked-changed version upload.			
Evidence			
We have used previous experience of assay development and research to provide these comments which I hope are helpful.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**NONE** No comments were uploaded or received from the user after several email attempts requesting their comments.		

Comment number	3		
Date received	06/09/2017	**Professional body**	Society for Applied Microbiology
Section	General		
Comment			
We have an updated logo, which is attached.			
Evidence			
Not completed.			
Financial barriers			
Not completed.			
Health benefits			
Not completed.			
Recommended action	**ACCEPT** This will be amended accordingly in all the UK SMI templates.		

Comment number	4		
Date received	13/09/2017	**Lab name**	PHE
Section	General		
Comment			

a. General comment on the style and readability of the document: This UK SMI contains a great deal of valuable advice and information. It would benefit however, from being edited into a single style. - For example, in paragraphs 3 & 4 of section 1.1 there is a mixture of the active and passive; please select one or the other. - Similarly, must and should are used almost interchangeably but in para 3 of section 2.1 a couple of instances of ‘shall’ creep in. Please ensure the appropriate meaning has been used.

b. Terminology: - In section 2.2, reference is made to 'PCR workstation laminar flow cabinet'. Please note that such cabinets may not all use laminar flow; some use HEPA-filtered air (non-laminar), while others use still air.

c. In section 6 where UK NEQAS is referred to, the word 'assurance' should be 'assessment' (according to the UK NEQAS web site).

d. Typos etc.: - Section 1.1, para 3. A 'policy' should be followed or observed; a 'procedure' or 'code of practice' may be practised.

e. Section 2.1. Suggest ‘However, this should not be into clean areas.'

f. Section 5, para 7. This is a statement only and needs to be re-phrased as a recommendation.

g. Quality management points:- Section 4, para 5. Where it is suggested that positive control material can be contrived specimens, the issue of commutability of the material could be included.

h. Section 5, para 8. In this reminder about the importance of document control two key points have been included (up to date and current) but the need for management approval/authorisation has been omitted.

i. Section 6, para 3. Assay validation is mentioned but equipment validation is not. That point could be inserted in section 5 para 5, to strengthen the importance of asset management.

j. Reagent management. In light of an imminent PHE 'lessons learnt' report on the quality of reagents in molecular assays, there is an opportunity to include some additional best practice points about selection of suppliers, more detail on adequacy of acceptance testing procedures, and separately emphasis on risk management principles (in relation to ISO 15189) and also, perhaps, the value of end-to-end bar coding of samples and electronic reporting of results.

Evidence

Not completed.

Financial barriers

No.

Health benefits

No.

Recommended action	a. **ACCEPT** This has been updated in the document accordingly. b. **ACCEPT** This has been updated in the document accordingly. c. **ACCEPT** This has been updated in the document accordingly. d. **NONE** This sentence will remain in the document as good housekeeping should be observed at all times in the laboratories. e. **ACCEPT** This has been updated in the document accordingly. f. **ACCEPT** This has already been updated in the document accordingly. g. **ACCEPT** This has been updated in the document accordingly. h. **ACCEPT** This has been updated in the document accordingly.

	i. **NONE** Equipment validation is already in the UK SMI Q1 – "Evaluations, validations and verifications of diagnostic tests" document which is linked in the paragraph of Section 6. This has also been briefly mentioned in paragraph 5 of Section 5 that equipment used should be calibrated periodically. j. **NONE** This is outside the remit of this UK SMI document.

Comment number	5		
Date received	13/09/2017	**Lab name**	North West London Pathology
Section	Title		
Comment			
This SOP seems mis-named as there is no mention of other molecular techniques except PCR - Suggest retitling the SMI - good practice when performing PCR-Based molecular amplification assays.			
Evidence			
Scope of document This UK SMI describes key elements of how to organise facilities for molecular amplification assays. Introduction The ability of PCR to produce large numbers of copies of a target sequence from minute quantities - sometimes single copies - of DNA has provided the exquisite sensitivity that makes PCR a powerful diagnostic tool. However, this ability also necessitates that extreme care needs to be taken to avoid the generation of false positive results.			
Financial barriers			
None.			
Health benefits			
No.			
Recommended action	**NONE** There is mention of Next Generation Sequencing technology.		

Comment number	6		
Date received	15/09/2017	**Lab name**	Royal Cornwall Hospital
Section	Several		
Comment			

We discussed this UK SMI with staff who do and don't work in Molecular. a. The novices asked if more information could be provided in the Introduction (e.g. the word PCR - what does it stand for; what is the difference between molecular and PCR testing?) b. Section 5, pg14 - perhaps more explanation on good pipette technique (ie smooth, downward pressure to reduce contamination/aerosols). c. Also what is 'regular' environmental swabbing - how often? d. No mention of having separate fridges in each room. If there reagents are kept in a fridge in the pre-PCR room and they are taken into another room, they cannot be returned.	
Evidence	
Not completed.	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	a. **ACCEPT** The acronym PCR has been written in full. b. **NONE** This is not within the remit of this UK SMI document. c. **NONE** The frequency of how environmental swabbing of areas where high throughput PCRs are performed is down to local policies. However, it is advisable to do so to avoid contamination. d. **NONE** This is not within the remit of this UK SMI document.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 5			
Date received	16/08/2017	**Lab name**	Virology, The James Cook University Hospital, Middlesbrough
Health benefits			

The use of good laboratory practice will have health benefits for staff working in the laboratory to protect both themselves and their colleagues from unnecessary exposure to potentially harmful pathogens.			
Date received	18/08/2017	**Lab name**	Senior clinical scientist
Health benefits			
No.			
Date received	21/08/2017	**Lab name**	Animal and Plant Health Agency
Health benefits			
No, as staff who work with zoonotic diseases will be on occupational health schemes.			
Date received	08/09/2017	**Lab name**	Antrim Area Hospital Microbiology Laboratory
Health benefits			
No.			
Date received	12/09/2017	**Lab name**	Sheffield Teaching Hospitals Microbiology
Health benefits			
No.			