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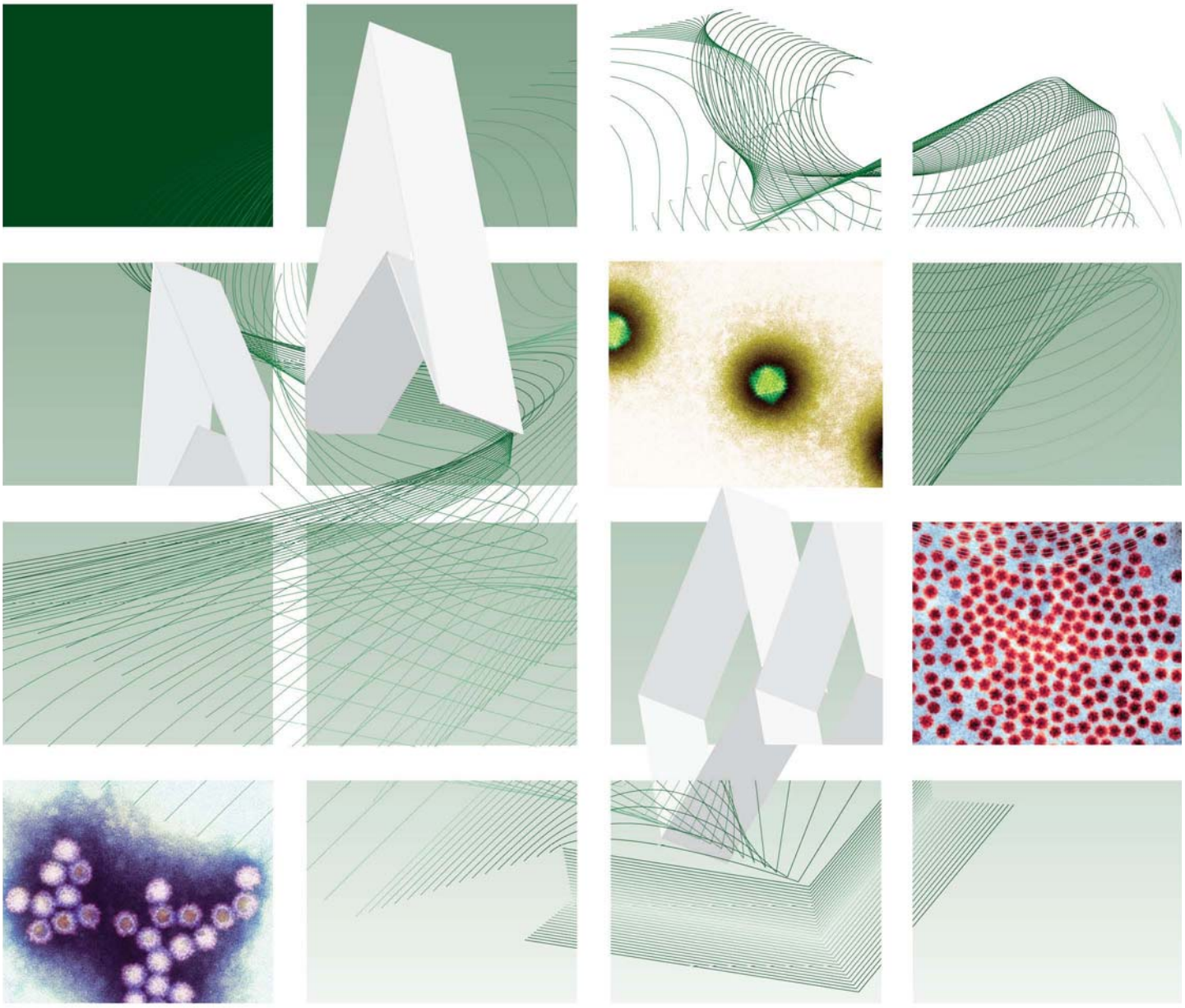
NHS

Health Protection Agency

UK Standards for Microbiology Investigations

Investigation of Swine-Lineage Influenza A/H1N1 v Using the Quadriplex Influenza A PCR Assay with Swine-Lineage Influenza A H1 and N1 Assays



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Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of the Health Protection Agency (HPA) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:

UK Standards for Microbiology Investigations[#]: Status

Users of SMIs

Three groups of users have been identified for whom SMIs are especially relevant:

- SMIs are primarily intended as a general resource for practising professionals in the field operating in the field of laboratory medicine in the UK. Specialist advice should be obtained where necessary.
- SMIs provide clinicians with information about the standard of laboratory services they should expect for the investigation of infection in their patients and the documents provide information that aids the electronic ordering of appropriate tests from hospital wards.
- SMIs also provide commissioners of healthcare services with the standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe essential laboratory methodologies which underpin quality, for example assay validation, quality assurance, and understanding uncertainty of measurement.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health interventions, surveillance, and research and development activities. SMIs align advice on testing strategies with the UK diagnostic and public health agendas.

Involvement of Professional Organisations

The development of SMIs is undertaken within the HPA in partnership with the NHS, Public Health Wales and with professional organisations.

The list of participating organisations may be found at http://www.hpa.org.uk/SMI/Partnerships. Inclusion of an organisation's logo in an SMI implies support for the objectives and process of preparing SMIs. Representatives of professional organisations are members of the steering committee and working groups which develop SMIs, although the views of participants are not necessarily those of the entire organisation they represent.

[#] UK Standards for Microbiology Investigations were formerly known as National Standard Methods.

Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

SMIs are developed, reviewed and updated through a wide consultation process. The resulting documents reflect the majority view of contributors. SMIs are freely available to view at http://www.hpa.org.uk/SMI as controlled documents in Adobe PDF format.

Quality Assurance

The process for the development of SMIs is certified to ISO 9001:2008.

NHS Evidence has accredited the process used by the HPA to produce SMIs. Accreditation is valid for three years from July 2011. The accreditation is applicable to all guidance produced since October 2009 using the processes described in the HPA's Standard Operating Procedure SW3026 (2009) version 6.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are well referenced and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. SMIs should be used in conjunction with other SMIs.

UK microbiology laboratories that do not use SMIs should be able to demonstrate at least equivalence in their testing methodologies.

The performance of SMIs depends on well trained staff and the quality of reagents and equipment used. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Whilst every care has been taken in the preparation of SMIs, the HPA, its successor organisation(s) and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

SMIs are the copyright of the HPA which should be acknowledged where appropriate.

Microbial taxonomy is up to date at the time of full review.

Equality and Information Governance

An Equality Impact Assessment on SMIs is available at http://www.hpa.org.uk/SMI.

The HPA is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

Suggested Citation for this Document

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Amendment Table

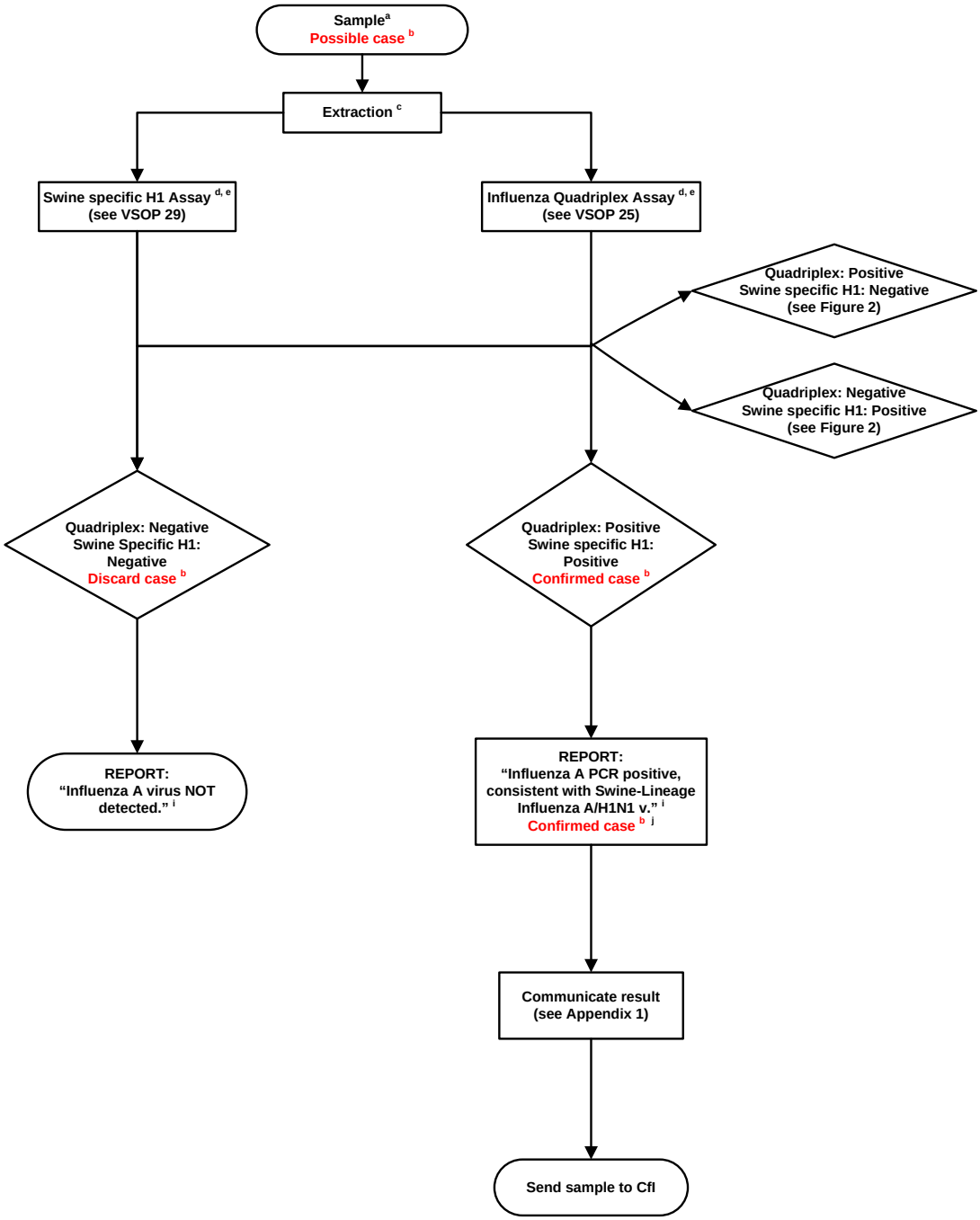
Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	2/02.11.11
Issue no. discarded.	1.1
Insert Issue no.	1.2
Section(s) involved.	**Amendment.**
Whole document.	Document presented in a new format.
References.	Some references updated.

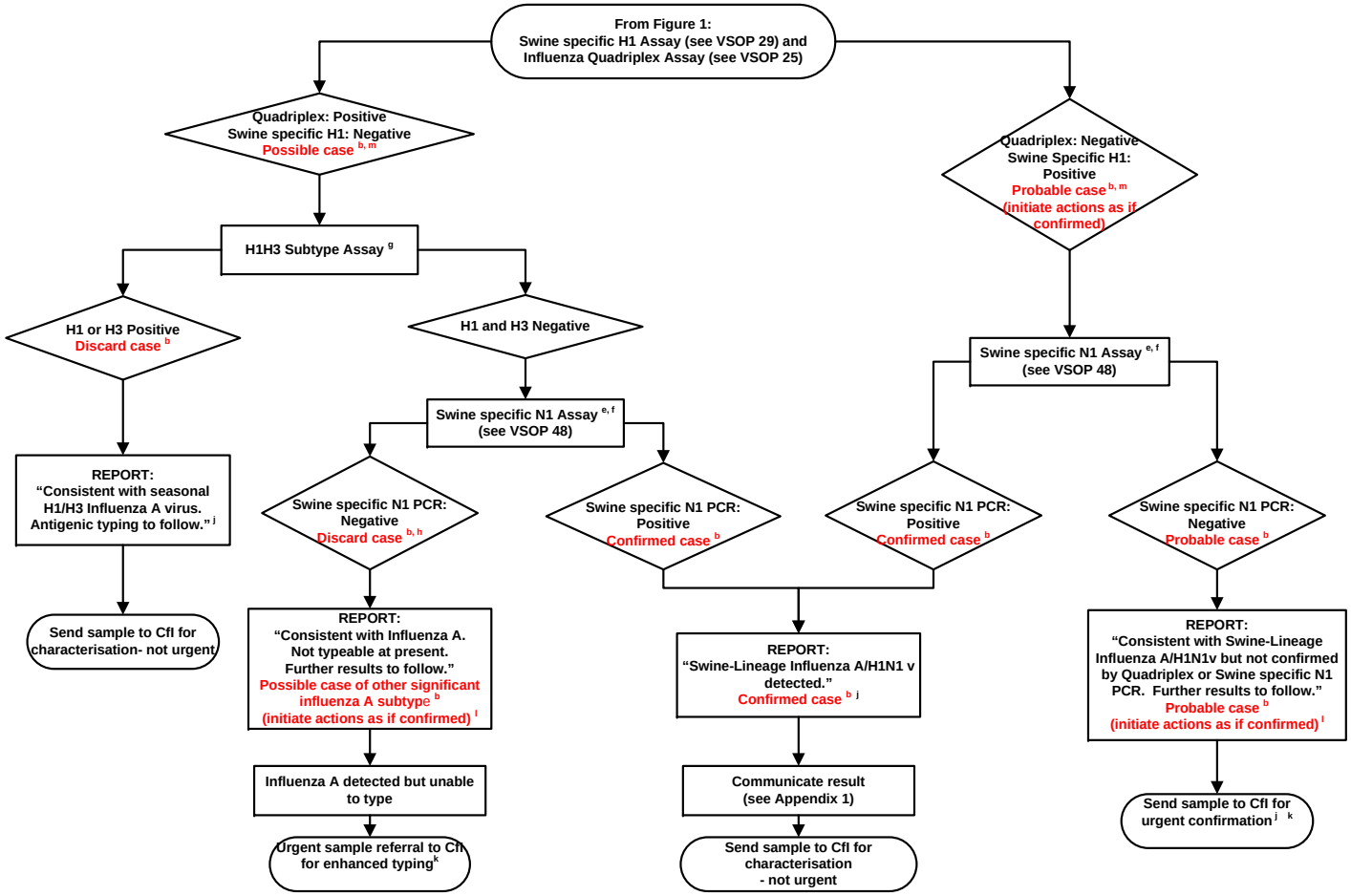
Amendment No/Date.	1/19.06.09
Issue no. discarded.	1
Insert Issue no.	1.1
Section(s) involved.	**Amendment.**
Figure 1.	Inactivation by heating removed from extraction box and placed in footnotes.
Figure 2.	Flow chart amended to make referrals easier. H1 or H3 positive report box amended to say antigenic testing.
Footnote c.	The need to keep aliquots of samples added.
Footnote m.	Footnote m added.
Appendix 2.	Appendix 2 and table added.

Figure 1: Investigation of Swine-Lineage Influenza A/H1N1 v Using the Quadriplex Influenza A PCR Assay with Swine-Lineage Influenza A H1 and N1 Assays[2]



NB: Changes in algorithm followed by the Devolved Administrations
Northern Ireland: Will use a Matrix Screening assay instead of Quadriplex assay. In addition to the HPA Swine specific assay they also perform the CDC assay.
Edinburgh and Glasgow: Will use a Matrix gene PCR multiplex assay instead of the Quadriplex assay. They will use a 'Dublin' swine specific assay, the CfI Swine specific assay will be used as second line assay.
Wales: Will use the NASBA flu A assay and confirm with the CDC and HPA Swine specific assays.

Figure 2



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Footnotes

a) Samples should be collected and sent to the laboratory in appropriate packaging (Class B packaging). Samples will usually be nose and throat swabs combined into virus transport medium (see HPA guidance on sample collection and transport: 'Standard practical advice for investigating individuals with possible swine influenza infection' available at www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1241048770758). Samples should be sent to a designated laboratory by approved courier. In the laboratory samples are to be treated as Hazard Group 2 specimens unless from known infected individuals, in which case they must be handled in a Class 1 or Class 2 Biological Safety Cabinet in a Category 2 laboratory (or at Category 3 containment if liquid samples such as sputum or NPA)[1].

b) This algorithm refers to investigation where the case definition is met or where surveillance for swine-lineage influenza A/H1N1 v has been undertaken. Case definitions are found on the HPA website as 'Swine Influenza case definition' at www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1241048739571. Definitions may change, so refer to above and to the latest algorithms. S6a algorithm on the HPA website http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1240732819361) and S6b http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1244023923195.

c) cA sample aliquot may be inactivated by heating at 80°C for 20 minutes. An aliquot should be retained for further characterisation at CfI including virus culture, should the initial RMN tests prove positive.

d) Swine-Lineage Influenza A H1 Specific Fast Real Time PCR – see V29 – Swine-Lineage Influenza A H1 specific fast Real Time PCR (available for password holders on request at www.hpa-standardmethods.org.uk) Note that the assay is validated for extraction using Qiagen QIAamp® Viral RNA Mini kit or Biomerieux NucliSENS® easy MAG.

   Real-time Influenza Quadriplex assay – see V 25 – Real-Time Quadriplex PCR for the detection of Influenza or similar generic influenza A matrix assay nucleic acid amplification test.

e) Note that the run control provided for the HPA real-time PCR assays is a genetically distinct positive control virus (A/Aragon/3218/2008); false positives due to contamination with this virus can be readily differentiated by sequencing from true positives.

f) Swine-lineage influenza A N1 real time confirmatory PCR assay – see V 48 (available for password holders on request at www.hpa-standardmethods.org.uk) or similar generic influenza A matrix assay nucleic acid amplification test.

g) One-Step Influenza Multiplex Real Time RT-PCR (Influenza A H1H3 Subtype assay) – see VSOP 50 (available for password holders on request at www.hpa-standardmethods.org.uk).

h) Arrange urgent transport of sample to CfI for characterisation as this pattern would be compatible with avian influenza (or human H2). Consider possibility of false positive Quadriplex (or similar generic influenza A matrix assay) or false negative swine-specific H1 assay and repeat, interpret in light of repeat test results. If unchanged on repeat await results from CfI.

i) Consider adding a comment such as 'A negative PCR does not necessarily exclude Swine-Lineage Influenza A/H1N1 v infection'. Tests can be falsely negative if: (i) Samples taken using incorrect swabs or transport medium; (ii) Samples taken poorly

(see sampling guidelines referred to in footnote b); (iii) Samples taken too early in the incubation period or too late in the recovery phase.

j) Report to CfI (OpsRoom), HPU and CDSC if Positive using fax or secure e-mail.

k) Current arrangements are to courier samples as soon as possible to CfI for confirmation (Respiratory Virus Unit, Virus Reference Department, HPA, CfI, Colindale, London, NW9 5HT. Telephone 020 8327 6239. Additional confirmatory testing may include sequencing.

l) Arrange urgent transport of sample to CfI for confirmation. Consider possibility of false negative Quadriplex or swine-specific N1 assay or false positive swine specific H1 assay, and repeat, interpret in light of repeat test results.

m) Report at this stage only if there will be significant delays in further testing - see Appendix 2.

Notification to the HPA[2,3]

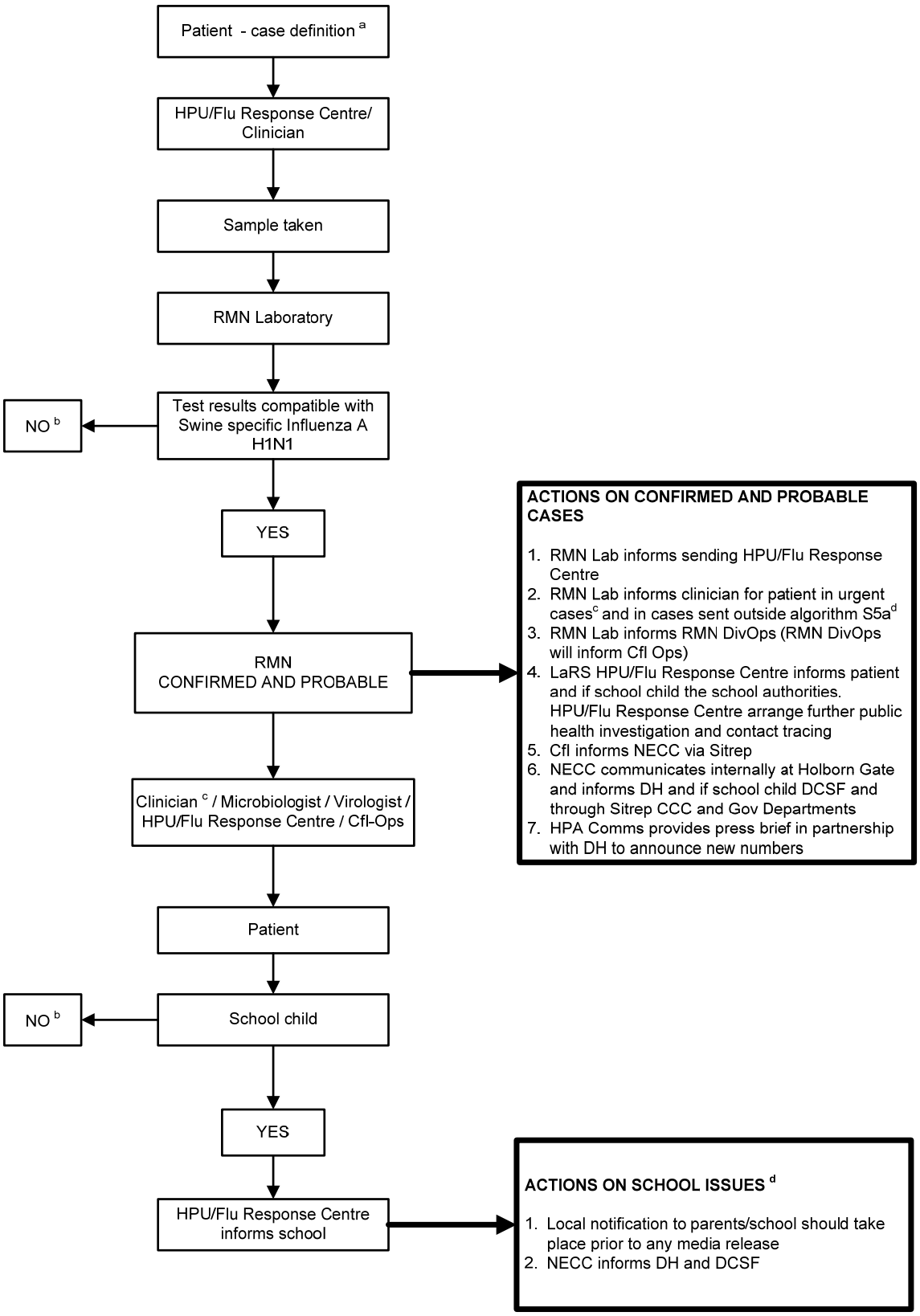
The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify the Health Protection Agency (HPA) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days. For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local HPA office. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to the HPA. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to the HPA and many HPA offices have agreements with local laboratories for urgent reporting of some infections. This should continue.

(Note: The Health Protection Legislation Guidance (2010) includes reporting of HIV & STIs, HCAIs and CJD under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories').

Other arrangements exist in Scotland[4] and Wales[5].

Appendix 1 – Management of Confirmed and Probable Cases



Appendix 2 – Swine Influenza A/H1N1 v Reporting Matrix

	Testing Sequence (left to right)						
Profile	Quadriplex (Matrix)	Swine H1	Swine N1	Subtype H1H3	Repeat Swine H1	Report	Action
1	Negative	Negative				Influenza A virus NOT detected	None
2	Positive	Positive				Influenza A virus PCR POSITIVE consistent with Swine lineage Influenza A/H1N1v. Confirmed case.	Communicate result. Send sample to CfI
3*	Positive	Negative				Influenza A virus PCR POSITIVE. Possible case. Further report to follow	Communicate result. Further testing locally
4	Positive	Negative		Positive		Influenza A virus PCR POSITIVE consistent with seasonal H1/H3 Influenza A virus. Swine lineage Influenza A/H1N1v virus NOT detected. Further results to follow.	Communicate result. Send sample to CfI
5	Positive	Negative	Positive	Negative		Influenza A virus PCR POSITIVE. Swine lineage Influenza A/H1N1v DETECTED. Case confirmed.	Communicate result. Send sample to CfI
6	Positive	Negative	Negative	Negative		Influenza A virus PCR POSITIVE consistent with Influenza A virus. Not typeable at present. Swine lineage Influenza A/H1N1v virus NOT detected. Possible case of other significant Influenza A virus subtype. Further results to follow.	Communicate result. Send sample to CfI URGENTLY
7*	Negative	Positive				Influenza A virus PCR POSITIVE consistent with Swine lineage Influenza A/H1N1v. Probable case. Further report to follow	Communicate result. Further testing locally
8	Negative	Positive	Positive			Influenza A virus PCR POSITIVE. Swine lineage Influenza A/H1N1v DETECTED. Case confirmed.	Communicate result. Send sample to CfI

9	Negative	Positive	Negative		Footnote "l"	Influenza A virus PCR results consistent with Swine lineage Influenza A/H1N1v virus BUT not confirmed by Quadriplex or Swine specific N1 PCR. Probable case of swine influenza. Further results to follow.	Communicate result. Send sample to CfI URGENTLY

Footnote

l Arrange urgent transport of sample to CfI for confirmation. Consider possibility of false negative Quadriplex or swine-specific N1 assay or false positive swine specific H1 assay, and repeat, interpret in light of repeat test results.

* These should be reported as interim results only where N1 or H1H3 testing will be significantly delayed

References

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