

NATIONAL STANDARD METHOD

INVESTIGATION OF SWINE-LINEAGE INFLUENZA A/H1N1 v USING THE QUADRIplex INFLUENZA A PCR ASSAY WITH SWINE-LINEAGE INFLUENZA A H1 AND N1 ASSAYS

VSOP 49

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections



UK Clinical Virology Network



Association of Medical Microbiologists
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INVESTIGATION OF SWINE-LINEAGE INFLUENZA A/H1N1 v USING THE QUADRIplex INFLUENZA A PCR ASSAY WITH
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The reader is informed that all taxonomy in this document was correct at time of issue.

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AMENDMENT PROCEDURE

Controlled document reference	VSOP 49
Controlled document title	Investigation of Swine-Lineage Influenza A/H1N1 v using the Quadriplex Influenza A PCR assay with Swine-Lineage Influenza A H1 and N1 assays

Each National Standard 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.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
1/ 19.06.09	1	1.1	5	Figure 1	Inactivation by heating removed from extraction box and placed in footnotes
			6	Figure 2	Flow chart amended to make referrals easier. H1 or H3 positive report box amended to say antigenic typing
			7	Footnote c	The need to keep aliquots of samples added
			8	Footnote m	Foot note m added
			11	Appendix 2	Appendix 2 and table added

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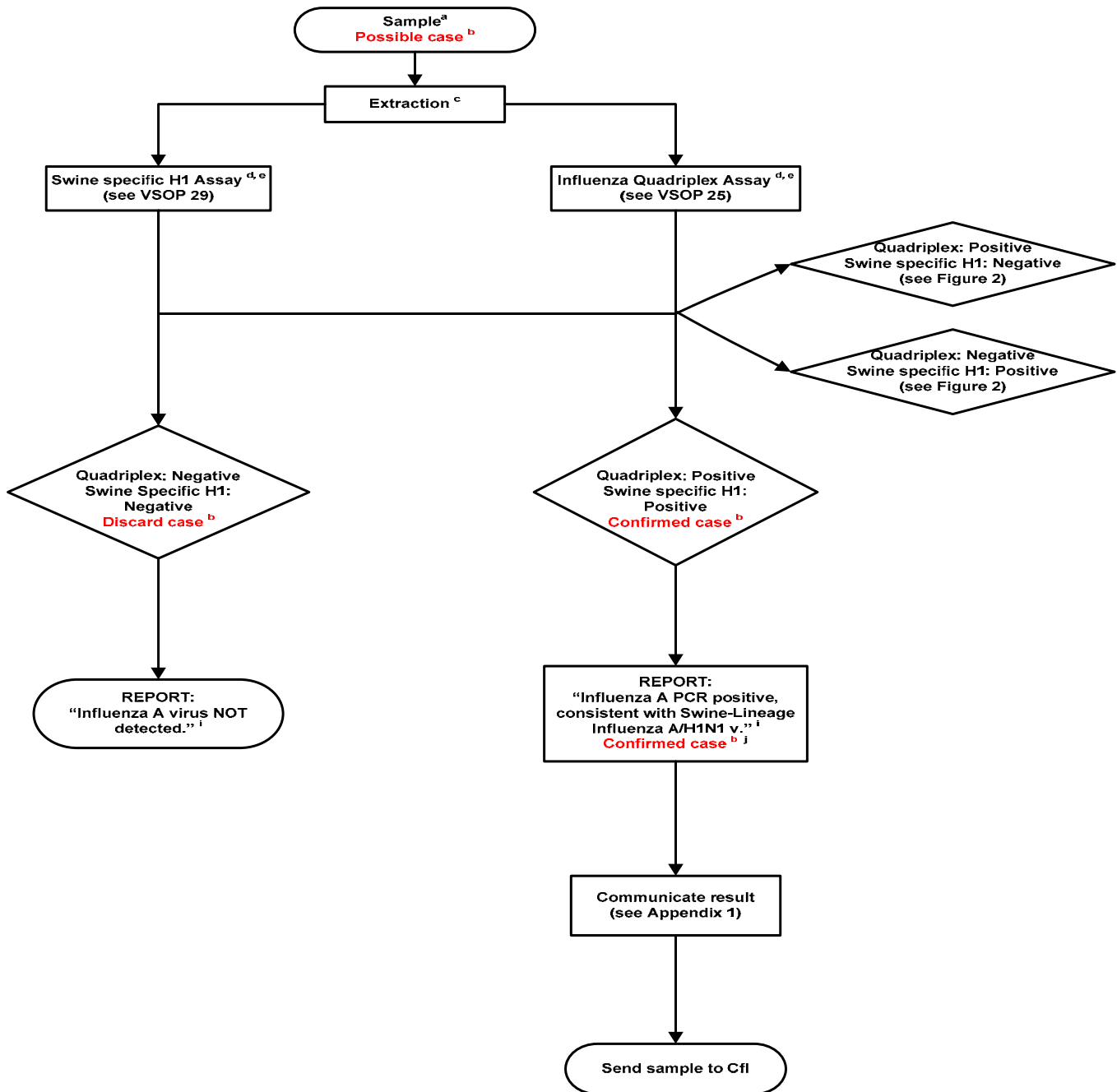
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FIGURE 1: INVESTIGATION OF SWINE-LINEAGE INFLUENZA A/H1N1 v USING THE QUADRIPLEX INFLUENZA A PCR ASSAY WITH SWINE-LINEAGE INFLUENZA A H1 AND N1 ASSAYS²



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.

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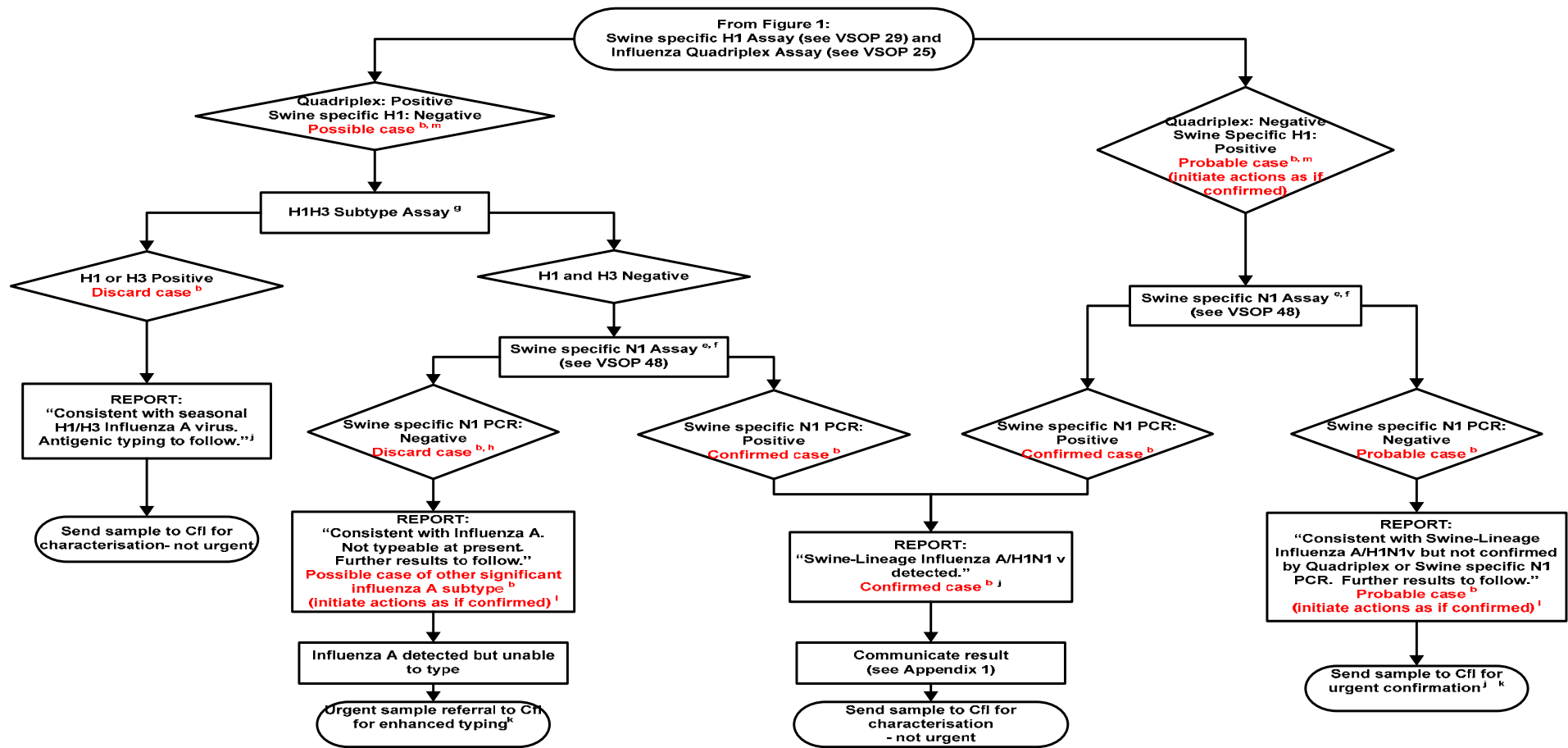
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FIGURE 2:



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 Cfl 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.

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- 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)²
- 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 A sample aliquot may be inactivated by heating at 80°C for 20 minutes. An aliquot should be retained for further characterisation at Cfl including virus culture, should the initial RMN tests prove positive.
- d Swine-Lineage Influenza A H1 Specific Fast Real Time PCR – see [VSOP29 – 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 [VSOP25 – 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 VSOP48](#) (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 Cfl 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 Cfl.

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- 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 Cfl (OpsRoom), HPU and CDSC if Positive using fax or secure e-mail
- k Current arrangements are to courier samples as soon as possible to Cfl for confirmation (Respiratory Virus Unit, Virus Reference Department, HPA, Cfl, Colindale, London, NW9 5HT. Telephone 020 8327 6239. Additional confirmatory testing may include sequencing.
- l Arrange urgent transport of sample to Cfl 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

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This National Standard Method has been developed, reviewed and revised by the Standard Methods Working Group for Clinical Virology (http://www.hpa-standardmethods.org.uk/wg_virology.asp). The contributions of many individuals in clinical virology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

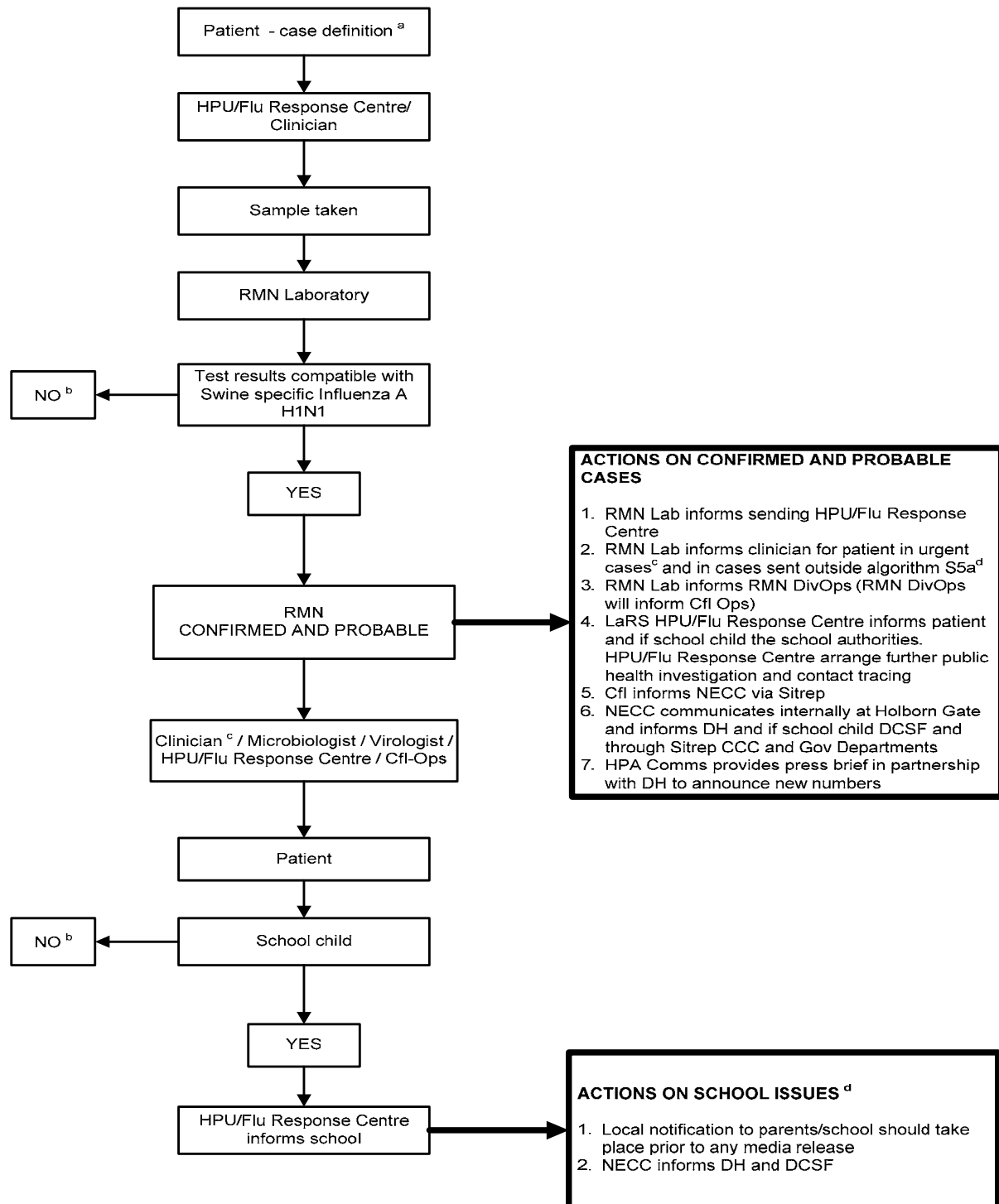
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APPENDIX 1 – MANAGEMENT OF CONFIRMED AND PROBABLE CASES



^a See HPA guidance as agreed with Regional Influenza Response Unit or HPU

^b Please refer to reporting algorithm for negative results

^c This may vary from region to region subject to local agreement with HPUs

^d Check guidance on HPA website

APPENDIX 2 – SWINE INFLUENZA A/H1N1 V REPORTING MATRIX

Profile	Testing Sequence (left to right)					Report	Action
	Quadruplex (Matrix)	Swine H1	Swine N1	Subtype H1H3	Repeat Swine H1		
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 Cfl
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. Antigenic typing results to follow.	Communicate result. Send sample to Cfl
5	Positive	Negative	Positive	Negative		Influenza A virus PCR POSITIVE. Swine lineage Influenza A/H1N1v DETECTED. Case confirmed.	Communicate result. Send sample to Cfl
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 Cfl 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 Cfl
9	Negative	Positive	Negative		Footnote "1"	Influenza A virus PCR results consistent with Swine lineage Influenza A/H1N1v virus BUT not confirmed by Quadruplex or Swine specific N1 PCR. Probable case of swine influenza. Further results to follow.	Communicate result. Send sample to Cfl URGENTLY

Footnote

¹ Arrange urgent transport of sample to Cfl for confirmation. Consider possibility of false negative Quadruplex 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.

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2. Advice on working with influenza viruses. <http://www.hse.gov.uk/biosafety/diseases/pandflu.htm>. p. 1-6.