

NATIONAL STANDARD METHOD

# INVESTIGATION OF SWINE-LINEAGE INFLUENZA A (H1N1) BY PCR

VSOP 47

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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# STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at [www.hpa.org.uk](http://www.hpa.org.uk).

The HPA aims to be a fully 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<sup>1</sup>.

More details can be found on the website at [www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk). Contributions to the development of the documents can be made by contacting [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

The reader is informed that all taxonomy in this document was correct at time of issue.

*Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.*

## **Suggested citation for this document:**

Health Protection Agency (2009). *Investigation of Swine-Lineage Influenza A (H1N1) by PCR*. National Standard Method VSOP 47 issue 3. [http://www.hpa-standardmethods.org.uk/pdf\\_sops.asp](http://www.hpa-standardmethods.org.uk/pdf_sops.asp).

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# AMENDMENT PROCEDURE

<b>Controlled document reference</b>	<b>VSOP 47</b>
<b>Controlled document title</b>	<b>Investigation of Swine-Lineage Influenza A (H1N1) by PCR</b>

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](mailto: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
3/ 03.06.09	2.1	3	5	<b>HPA swine influenza testing service</b>	Flowchart removed
			6	<b>All</b>	Red text moved below black text in algorithm
			8	<b>Appendix 1</b>	Actions on confirmed and probable cases point two amended

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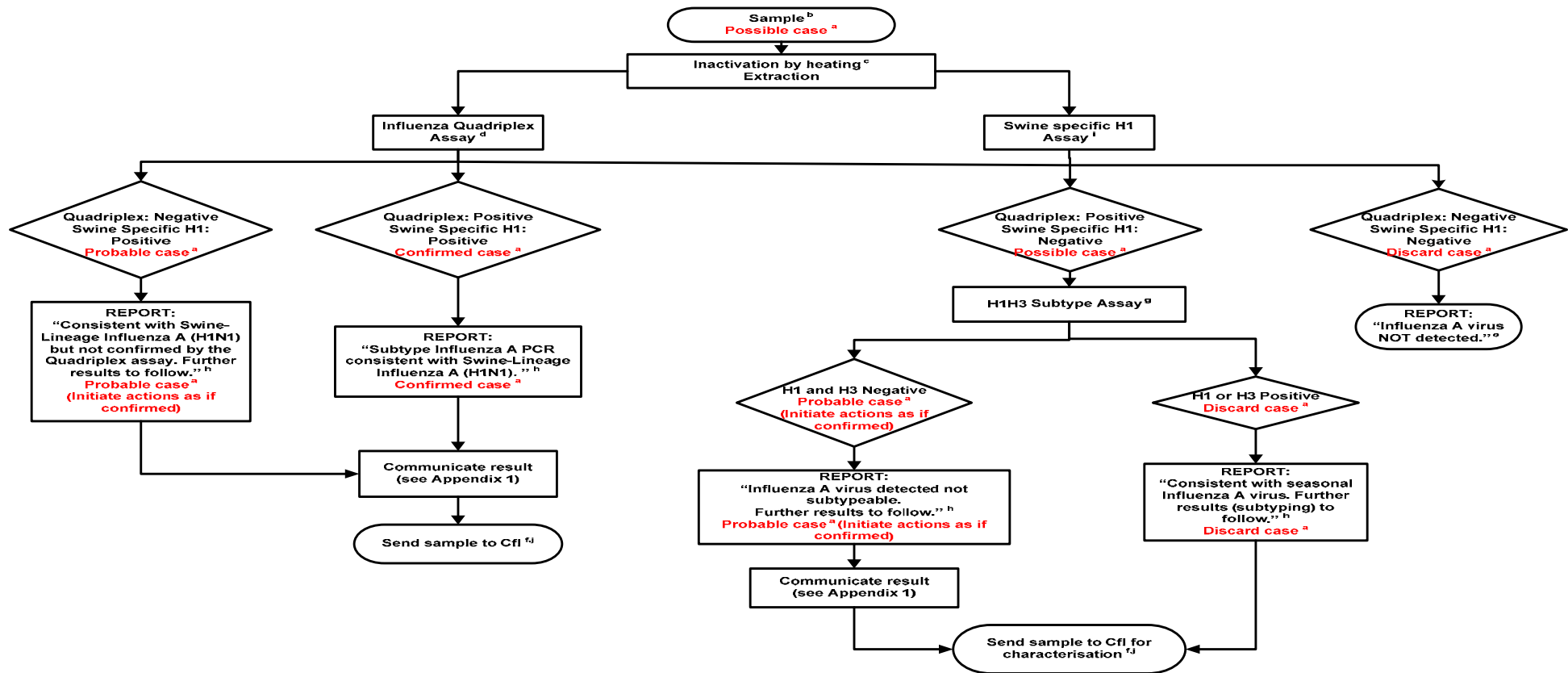
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# INVESTIGATION OF SWINE-LINEAGE INFLUENZA A (H1N1) <sup>2</sup>



**NB: Changes in algorithm followed by the Devolved Administrations**

**Northern Ireland:** Will use a Matrix Screening assay instead of Quadruplex 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 Quadruplex 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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- a This algorithm refers to investigation where the case definition is met or where surveillance for swine-lineage influenza A/H1N1 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](http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1241048739571). Definitions may change, so refer to above and to the latest algorithm S5 on the HPA website [www.hpa.org.uk/web/HPAwebFile/HPAweb\\_C/1240732819361](http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1240732819361))
- b 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](http://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)<sup>2</sup>
- c Samples may be inactivated by heating at 80°C for 20 minutes.
- d Real-time Influenza Quadriplex assay – see [VSOP25 – Real-Time Quadriplex PCR for the detection of Influenza](#) (available for password holders on request at [www.hpa-standardmethods.org.uk](http://www.hpa-standardmethods.org.uk)) or similar generic influenza A matrix assay nucleic acid amplification test.
- e Consider adding a comment such as 'A negative PCR does not necessarily exclude Swine-Lineage Influenza A (H1N1) 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.
- f 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
- g One-Step Influenza Multiplex Real Time RT-PCR (Influenza A H1H3 Subtype assay) – see V-5413/02-07 (available via [www.hpa-bioinformatics.org.uk](http://www.hpa-bioinformatics.org.uk))
- h Report to Cfl (OpsRoom), HPU and CDSC if Positive using fax or secure e-mail
- i 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](http://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
- j Additional confirmatory testing may include swine-specific N1 PCR and sequencing

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# ACKNOWLEDGEMENTS AND CONTACTS

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](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.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

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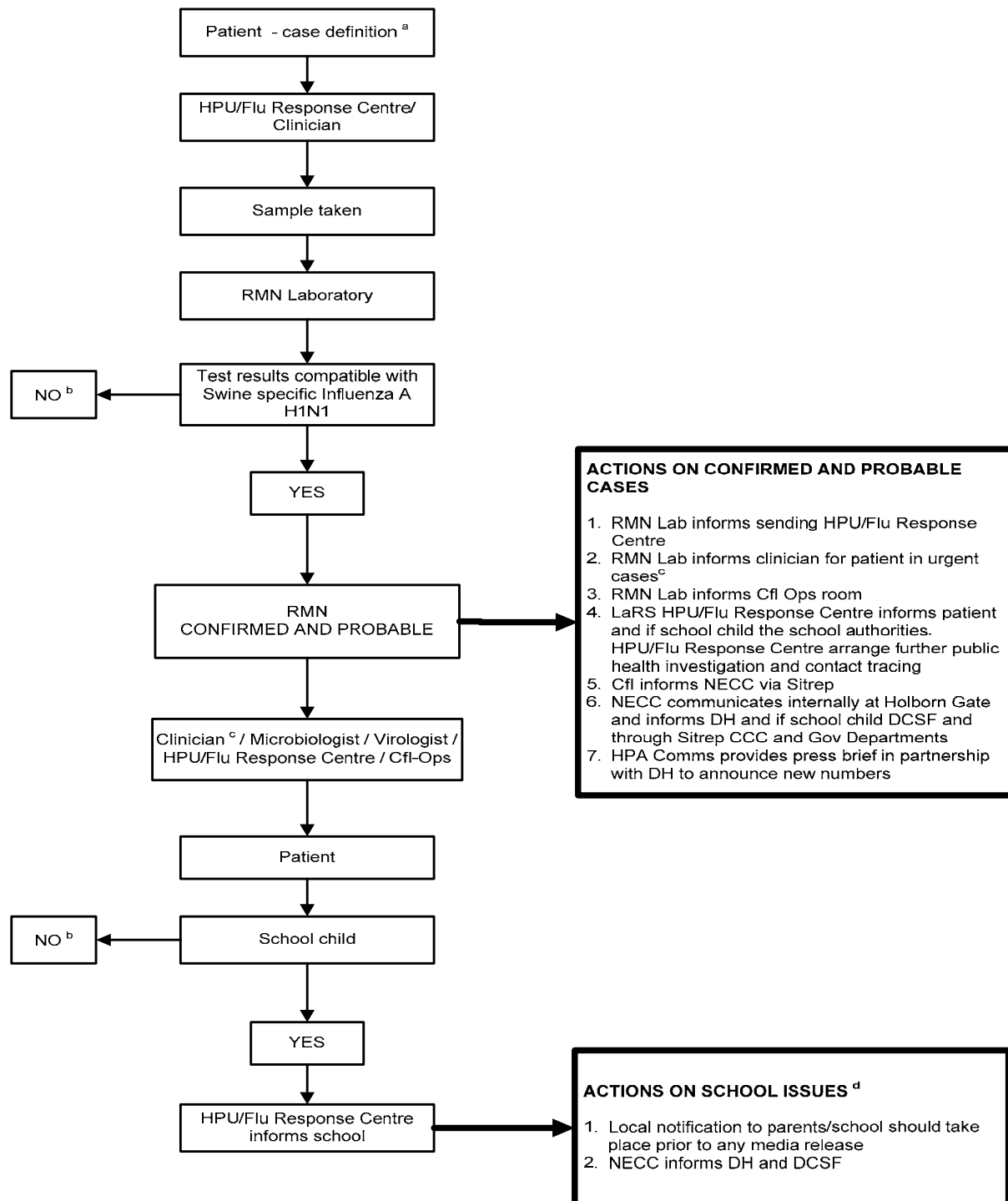
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# APPENDIX 1 – MANAGEMENT OF CONFIRMED AND PROBABLE CASES



<sup>a</sup> See HPA guidance as agreed with Regional Influenza Response Unit or HPU

<sup>b</sup> Please refer to reporting algorithm for negative results

<sup>c</sup> This may vary from region to region subject to local agreement with HPUs

<sup>d</sup> Check guidance on HPA website

## INVESTIGATION OF SWINE-LINEAGE INFLUENZA A (H1N1) BY PCR

# REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.
2. Advice on working with influenza viruses. <http://www.hse.gov.uk/biosafety/diseases/pandflu.htm>. p. 1-6.

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