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# Note from the editors: A new virus bringing back memories from the past

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In recent days, public health experts and healthcare workers around the world are alert following the discovery of a new human coronavirus causing severe respiratory illness. Two cases, both with connection to Saudi Arabia, were communicated through ProMED on 20 and 23 September respectively [1,2].

Many health professionals still have vivid memories of the alert that followed the death of an American businessman in a hospital in Hanoi, Vietnam, in early 2003 after having travelled to China, and the following outbreak of severe acute respiratory syndrome (SARS). This triggered worldwide alarm and containment measures. During the outbreak, there was excellent collaboration between global players and institutions, on various levels (i.e. public health institutions, laboratories and hospitals) and new ways of communicating proved to be highly value for the exchange of information. The last case of SARS occurred in China in May 2004: thereafter the virus seemed to have disappeared and has not resurfaced since.

The public health world is currently looking closely into the two recent cases of coronovirus infection. Similar to SARS, the two patients had/have symptoms of severe respiratory illness and the virus comes from the same family, *Coronaviridae*. However, there are some marked differences. The virus is not the same: laboratory analyses have proven that the new virus is not a SARS-like virus. Furthermore, the two confirmed cases occurred with a gap of three months between them and there is no evidence of a direct epidemiological link.

Much remains unknown at the moment and information that would allow us to make a final judgment about the disease is missing. Two rapid communications in this issue give a timely account of the recommended public health measures and assays to detect the virus. On the basis of the limited evidence currently available, the risk for person-to-person transmission, as assessed by the European Centre for Disease Prevention and Control (ECDC) in a rapid risk assessment, is considered low [3]. *Eurosurveillance* will continue to provide more information as it becomes available.

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# Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction

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We present two real-time reverse-transcription polymerase chain reaction assays for a novel human coronavirus (CoV), targeting regions upstream of the E gene (upE) or within open reading frame (ORF)1b, respectively. Sensitivity for upE is 3.4 copies per reaction (95% confidence interval (CI): 2.5–6.9 copies) or 291 copies/mL of sample. No cross-reactivity was observed with coronaviruses OC43, NL63, 229E, SARS-CoV, nor with 92 clinical specimens containing common human respiratory viruses. We recommend using upE for screening and ORF1b for confirmation.

## Introduction

Coronaviruses (CoV) are large positive-stranded RNA viruses causing mainly respiratory and enteric disease in a range of animals and in humans. Humans are known to maintain circulation of four different human coronaviruses (hCoV) at a global population level. These are part of the spectrum of agents that cause the common cold. The SARS-CoV constitutes a fifth hCoV, which was in circulation for a limited time during 2002 and 2003, when a novel virus appeared in humans and caused an outbreak affecting at least 8,000 people. Mortality was high, at ca. 10% [1]. Symptoms matched the clinical picture of acute primary viral pneumonia, termed severe acute respiratory syndrome (SARS).

During September 2012, health authorities were notified of two cases of severe hCoV infection caused by a novel virus type. Both patients had travelled, or resided, in Saudi Arabia. Laboratories dealing with each of these unlinked cases were situated in Jeddah, Rotterdam and London, respectively.

In a collaborative activity co-ordinated by major European and national epidemic response networks we have developed diagnostic real-time reverse-transcription polymerase chain reaction (RT-PCR) assays suitable for qualitative and quantitative detection of the new agent. Here we summarise the technical evaluation and analytical performance of these assays.

## Materials and methods

### Template for design of assays

A provisional genome sequence as well as an isolate of the new virus were obtained from author RM Fouchier on 24 September 2012, after public notification of the second case case, who was in the United Kingdom (UK), to be most probably infected by the same virus as the first case, yet unrelated. The sequence (GenBank accession number: JX869059 for the Rotterdam virus isolate, termed hCoV-EMC) served as the template for assay design, and the virus was used for initial validation experiments.

### Clinical samples

Respiratory swab, sputum, and endotracheal aspirate material was obtained during 2010–2012 from several hospital wards of the University of Bonn Medical Centre.

### Cell culture

Vero cells were infected with a the cell culture isolate (unpublished data) at two different doses (multiplicities of infection (MOI) of ca. 0.1 and ca. 10 TCID50 per cell) and harvested after 0, 12, 24, and 36 hours for RT-PCR analysis.

### RNA extraction

RNA was extracted from the samples as described earlier [2] by using a viral RNA mini kit (Qiagen). Sputum samples were pretreated with 2× sputum lysis buffer (10 g of N-acetylcysteine/litre, 0.9% sodium chloride) for 30 minutes in a shaking incubator. Swabs were immersed in lysis buffer.

## Real-time reverse-transcription polymerase chain reaction screening assay upstream of E gene (upE assay)

A 25-µl reaction was set up containing 5 µl of RNA, 12.5 µl of 2 X reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen; containing 0.4 mM of each dNTP and 3.2 mM Magnesium sulfate), 1 µl of reverse transcriptase/Taq mixture from the kit, 0.4 µl of a 50 mM magnesium sulfate solution (Invitrogen – not provided with the kit), 1 µg of non-acetylated bovine serum albumin (Sigma), 400 nM concentrations of primer upE-Fwd (GCAACGCGCGATTCAGTT) and primer upE-Rev (GCCTCTACACGGGACCCATA), as well as 200 nM of probe upE-Prb (6-carboxyfluorescein [FAM])-CTCTTCACATAATCGCCCCGAGCTCG-6-carboxy-N,N,N,N´-tetramethylrhodamine [TAMRA]). All oligonucleotides were synthesized and provided by Tib-Molbiol, Berlin. Thermal cycling involved 55°C for 20 min, followed by 95°C for 3 min and then 45 cycles of 95°C for 15 s, 58°C for 30 s.

It should be mentioned that common one-step real-time RT-PCR kits formulated for application with probes should all provide satisfactory results with default reaction mix compositions as suggested by manufacturers. In the particular case of our formulation the bovine serum albumin can be omitted if using a PCR instrument with plastic tubes. The component only serves the purpose of enabling glass capillary-based PCR cycling.

## Real-time reverse-transcription polymerase chain reaction confirmatory assay (open reading frame (ORF)1b gene)

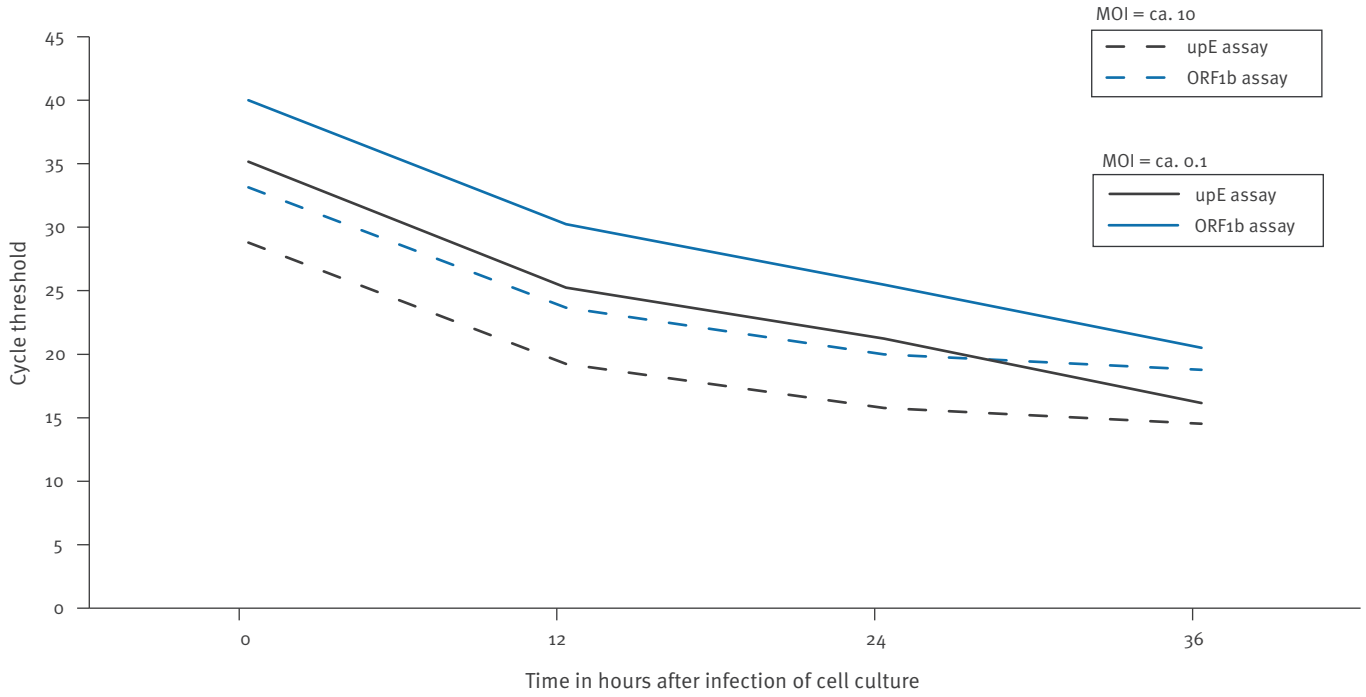
The assay had the same conditions as for the upE RT-PCR, except primer and probe sequences were ORF1b-Fwd (TTCGATGTTGAGGGTGCTCAT), primer ORF1b-Rev (TCACACCAGTTGAAAATCCTAATTG), and probe ORF1b-Prb (6-carboxyfluorescein [FAM])- CCCGTAATGCATGTGGCACCAATGT-6-carboxy-N,N,N,N´-tetramethylrhodamine [TAMRA]). This target gene did not overlap with those of known pan-CoV assays [3-5].

## In-vitro transcribed RNA controls

PCR fragments covering the target regions of both assays, and some additional flanking nucleotides ('peri-amplicon fragments'), were generated using primers CTTCTCATGGTATGGTCCCTGT and AAGCCATACACACCAAGAGTGT for the upE assay, and CGAGTGATGAGCTTTGCGTGA and CCTTATGCATAAGAGGCACGAG for the ORF1b assay. Products were ligated into pCR 4 plasmid vectors and cloned in *Escherichia coli* by means of a pCR 4-TOPO TA

**Figure 1**

Replication of hCoV-EMC monitored by the upE and ORF1b RT-PCR assays, 2012




MOI : mulitplicity of infection (TCID50 per cell); RT-PCR: real-time reverse transcription-polymerase chain reaction; upE: upstream of the E gene; ORF1b: open reading frame 1b gene.

Vero cells were infected with hCoV-EMC at two different doses (MOI: ca. 10 and MOI: ca. 0.1) and standardised samples taken at different time points (after 0, 12, 24, and 36 hours) were tested by the upE and ORF1b RT-PCR assays.

cloning reagent set (Invitrogen). Plasmids were examined for correct orientation of inserts by PCR, purified, and re-amplified with plasmid-specific primers from the reagent set to reduce the plasmid background in subsequent in vitro transcription. Products were transcribed into RNA with the MegaScript T7 in vitro transcription reagent set (Ambion). After DNase I digestion, RNA transcripts were purified with Qiagen RNeasy columns and quantified photometrically. All transcript dilutions were carried out in nuclease-free water containing 10 µg/mL carrier RNA (Qiagen).

## Determination of analytical sensitivities of real-time reverse-transcription polymerase chain reaction methods

Series of eight parallel reactions per concentration step were prepared and tested by the respective RT-PCR to determine concentration-dependent hit rates. Hit rates were subjected to probit regression analysis in StatgraphicsPlus software (version 5.0; Statistical Graphics Corp.).

## Specificity of the assays

Assay specificity was determined using high-titred virus stock solutions, as well as clinical samples known to contain respiratory viruses. All material stemmed from the in-house strain and sample collection of University of Bonn, Institute of Virology. Identities and virus RNA concentrations were re-confirmed by specific real-time RT-PCRs for each virus before the experiment. Measured RNA concentrations are listed below along with the recorded stock virus titres.

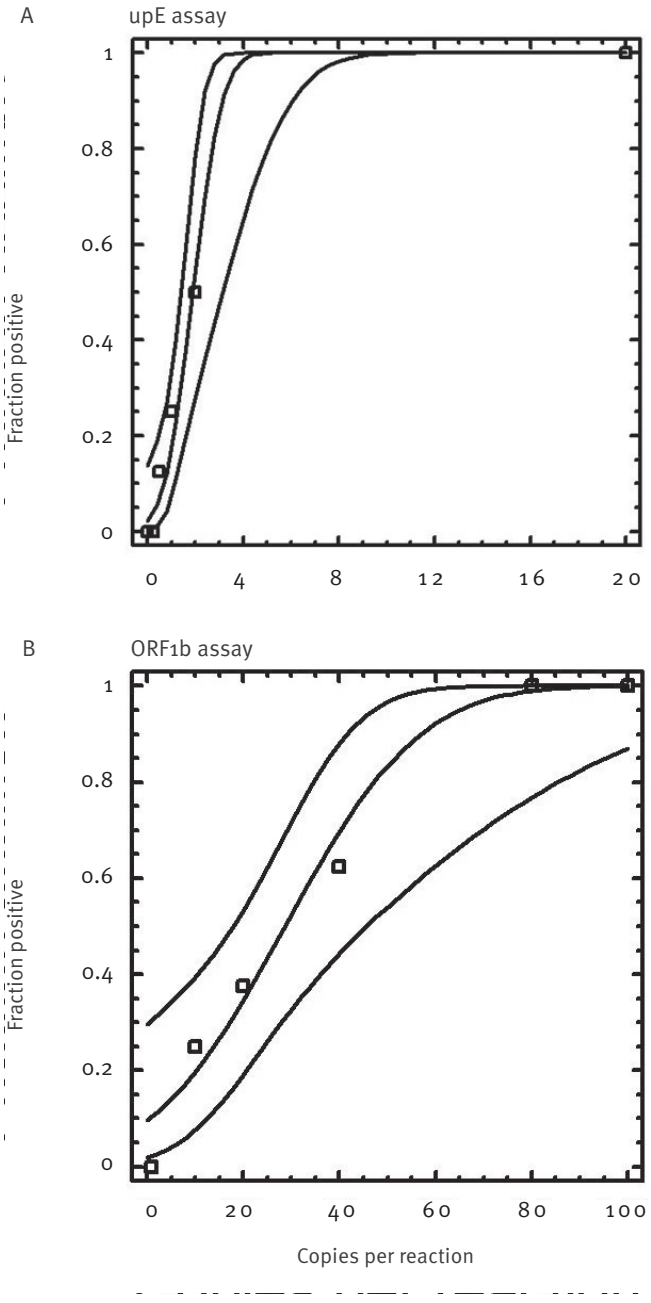
## Results

Upon scanning of a provisional genome assembly, a region upstream of the putative E gene was identified as a particularly suitable target region for a real-time RT-PCR assay. The assay designed for this region is hereafter referred to as the upE-assay. A confirmatory test was designed in the open reading frame 1b (termed the ORF1b assay). This target gene did not overlap with those of known pan-CoV assays [3-5].

In order to obtain an estimate of the end point sensitivity of the assays, they were applied to cell culture-derived virus stock. The virus had a titre of 1.26 x $10^7$ median tissue culture infective dose (TCID50)/mL. In limiting dilution experiments, the upE and ORF1b assays detected down to 0.01 and 0.1 TCID50 per reaction, respectively. The discrepancy between assays might be due to release of subgenomic RNA after onset of cytopathogenic effect (CPE) in cell culture, including the upE target fragment. As shown in Figure 1, PCRs on these samples indicated no divergence between the assays after onset of CPE (observed at 24h onwards). However, both assays deviated from each other by constant numbers of Ct values over the full duration of incubation, including time 0 (T0) when the cells were just infected and when no subgenomic RNA could have been present. It was concluded that the higher Ct values at each time point, and the lower dilution end point

**Figure 2**

Probit regression analysis to determine limit of detection for the upE and ORF1b assays, 2012




ORF: open reading frame of the frame; upE: upstream of the E gene.

The y-axis shows fractional hit-rates (positive reactions per reactions performed), the x-axis shows input RNA copies per reaction. Squares are experimental datum points resulting from replicate testing of given concentrations in parallels assays. The middle regression line is a probit curve (dose-response rule). The outer lines are 95% confidence intervals.

**Table 1**

Results of sensitivity and specificity tests for hCoV-EMC assays, 2012*

| Experiment | upE assay | ORF1b assay |
|---|---|---|
| Detection end point for cell culture-derived virus | 0.01 TCID50/reaction | 0.1 TCID50/reaction |
| Technical LOD | 3.4 RNA copies/reaction (95% CI: 2.5–6.9 copies/reaction) | 64 RNA copies/reaction (95% CI: 47–126 copies/reaction) |
| Cross-reactivity with hCoV-229E | No reactivity with virus containing $10^5$ PFU/mL ($3 \times 10^9$ RNA copies/mL) | |
| Cross-reactivity with hCoV-NL63 | No reactivity with virus containing $10^6$ PFU/mL ($4 \times 10^9$ copies/mL) | |
| Cross-reactivity with hCoV-OC43 | No reactivity with virus containing $5 \times 10^5$ PFU/mL ($3 \times 10^{10}$ copies/mL) | |
| Cross-reactivity with SARS-CoV | No reactivity with virus containing $3 \times 10^6$ PFU/mL ($5 \times 10^{10}$ copies/mL) | |

CI: confidence interval CoV: corona virus; LOD: limit of detection; ORF: open reading frame; PFU: plaque forming units; TCID50: median tissue culture infective dose; upE: upstream of the E gene.

for the ORF1b assay indicated that this assay had a lower sensitivity.

A more detailed assessment of technical sensitivity can be achieved using quantified, in-vitro transcribed RNA derived from the peri-amplicon region of each assay. These transcripts were generated and tested in serial ten-fold dilution experiments. Detection end points were two copies per reaction for the upE assay, and 10 copies per reaction for the confirmatory, ORF1b gene, assay. To obtain a statistically robust assessment of Limit Of Detection (LOD), transcripts were also tested in multiple parallel reactions in smaller dilution intervals above and below the end-point PCR limits. The results in terms of the fraction of positive reactions at each concentration were subjected to probit regression analysis and plotted as shown in Figure 2, where panel A shows the upE assay and panel B the ORF1b assay. The resulting LODs are summarised in Table 1. Based on the upE assay with a detection limit of 3.4 copies per reaction, and a cell-culture endpoint equivalent to 0.01 TCID50 per reaction, it was calculated that the RNA/infectious unit ratio of the virus stock must have been ca. 29 (100/3.4).

To exclude non-specific reactivity of oligonucleotides among each other, all formulations were tested 40 times in parallel with assays containing water and no other nucleic acids except the provided oligonucleotides. In none of these reactions was any positive signal seen. Cross-reactivity with known, heterospecific human CoVs was excluded by testing high-titred cell culture materials as summarised in Table 1. It should be noted that the unculturable hCoV-HKU1 was not included in these experiments.

To obtain a more clinically relevant figure on assay specificity, the assays were applied on 92 original clinical samples in which other respiratory viruses had already been detected during routine respiratory screening at Bonn University Medical Centre. These samples were prepared using the Qiagen Viral RNA kit, a formulation widely used to extract RNA in clinical laboratories. Of note, the tested panel included four samples containing hCoV-HKU1, which was not available as cultured virus stock. In total, none of the 92 original clinical samples as presented in Table 2, containing a wide range of respiratory viruses, gave any detection signal with either assay while positive controls were readily detected. It was concluded that the assay could be reliably applied to clinical samples.

Preliminary testing was also done on a patient hospitalised with acute infection during preparation of this report (Authors R Gopal and M Zambon, own unpublished observations). Both assays provided very clear amplification signal on various clinical samples. The upE assay again appeared more sensitive than the ORF1b assay.

## Discussion

Here we provide the technical background data for RT-PCR assays developed in rapid response to the emergence of a novel human CoV (GenBank accession number: JX869059 for the Rotterdam virus isolate, termed hCoV-EMC).

Cell culture-derived virus is a useful source of reference material for the evaluation of molecular detection assays. However, detection end points determined on cell culture-derived virus are difficult to correlate to virus titre. Reasons include the discrepancy between

infectious viral particles and the number of copies of viral RNA, as well as the imbalance between viral genomic and subgenomic transcripts in the particular case of CoVs. This is important for laboratories using cell-cultured virus as reference, but also in the clinical setting. For example, SARS-CoV assays targeting structural protein genes tend to be slightly more sensitive than ORF1b-based assays when applied to clinical samples [6]. For the novel virus the ratio of RNA copies per infectious unit was ca. 29, while little imbalance seems to exist between genomic and subgenomic RNA in Vero cells up to 36 h post infection.

While we are not addressing the issue of quantitative PCR in this report, it should be mentioned that the availability of synthetic RNA standards enables immediate implementation of quantitative virus detection that is essential for case management and public health. Quantitative virus data can help assess the height and duration of virus excretion, and can also be useful as an early and robust parameter for the success of treatment [2,7,8]. Here we have used synthetic RNA to determine technical limits of detection in the style of standards applied by industry, taking inter-assay variation into account and providing statistically robust detection end points based on physically quantified target genes, which is impossible to achieve on cell-cultured virus. It is important to note that the detection limits we describe here are expressed as copies per reaction. We have chosen not to translate these numbers into other terms such as 'copies per ml of sputum', 'copies per swab sample', or 'copies per gram of faeces'. Such transformations vary greatly between different RNA extraction methods and clinical materials. However, we can project that the level of sensitivity, particularly for the upE assay, is very similar to those levels achieved with most advanced RT-PCR assays developed for the SARS-CoV [6,8]. For example, the Qiagen Viral RNA kit with an input volume of 140 µl of sample and an elution volume of 60 µl as recommended by the manufacturer involves a conversion factor of 85.7 between copies per reaction and copies per mL of sample. The upE assay should thus detect as little as ca. 291 copies per mL of sputum with 95% certainty. For solid samples such as swabs, which can be dipped into the lysis buffer, the resulting conversion factor is 12, resulting in a projected capability of the assay to detect as little as ca. 41 copies per swab with 95% certainty.

In this regard it is highly important to remember practical experiences made with SARS-CoV detection. Even with the highest levels of RT-PCR sensitivity it turned out that not all patients retrospectively shown to seroconvert could be diagnosed by RT-PCR in the acute phase of disease [6,8,9]. This has been ascribed to the fact the SARS-CoV replication occurs predominantly in the lower respiratory tract due to the anatomical localisation of its entry receptor, Angiotensin-converting enzyme 2 (ACE2). Should the novel virus use the same receptor, we might see a similar distribution of virus, and similar challenges in clinical application of molecular diagnostics. Studies of virus concentration in clinical samples are underway to address these highly critical issues.

**Table 2**

Known respiratory viruses in clinical samples used for testing the specificity of hCoV-EMC assays, 2012

| Virus | Number of samples tested |
|---|---|
| Parainfluenza virus | |
| Parainfluenza 1 virus | 5 |
| Parainfluenza 2 virus | 5 |
| Parainfluenza 3 virus | 8 |
| Parainfluenza 4 virus | 1 |
| Respiratory syncytial virus | 7 |
| Human metapneumovirus | 8 |
| Coronavirus | |
| hCoV-NL63 | 6 |
| hCoV-OC43 | 4 |
| hCoV-229E | 2 |
| hCoV-HKU1 | 4 |
| Rhinovirus | 8 |
| Enterovirus | 9 |
| Adenovirus | 8 |
| Human Parechovirus | |
| Type 1 | 5 |
| Type 3 | 3 |
| Influenza A ($H_1N_1$, $H_3N_2$) | 9 |
| Influenza B | 2 |
| **Total** | **92** |

Specificity is a very important issue in rare, highly critical virus infections for which a broad number of differential diagnoses exist. The risk associated with false positive PCR results posed a challenge in development of the assays described here. First, real-time PCR can yield artificial signals due to technical interference of oligonucleotides involved in the assay (resembling primer dimers in which probe sequences participate). These may be observed at infrequent intervals due to the statistical nature of nonspecific random molecular interactions. We have taken care to exclude the occurrence of those signals by testing large series of water-containing assays. Second, any virus detection assay might cross-react with related viruses, and there is worldwide circulation of four different human CoVs. Viral stock solutions were tested in order to exclude cross-reactivity even on high-titred materials. In spite of the favourable outcome of this experiment, it should

be mentioned that of the two assays investigated, the target gene of the ORF1b-based assay was most conserved between CoV. The genetic range of known CoV from animals is larger than those human viruses tested here. Theoretical comparisons between genomes of these viruses and our ORF1b assay suggested no risk of significant cross-reactivity (not shown). However, in absence of further investigation we tend to recommend using the upE assay for case management. This is also due to the lower sensitivity of the ORF1b assay.

The final proof of assay specificity was provided in a set of clinical samples that was assembled to realistically reflect the composition of patient groups presenting with Acute respiratory infections (ARI). Of note, also the four 'common-cold coronaviruses' hCoV-NL63, -229E, -OC43, and -HKU1 were included in this panel. Consequentially, we can say from these data that typical human CoV will not cross-react with the assay, even under adverse conditions such as those created by the additional presence of patient-derived nucleic acid and other components typical of clinical samples that may all interfere with the performance of PCR.

The open availability of proven diagnostic assays early in an epidemic is useful in order to equip and prepare public health laboratories efficiently [10,11]. However, there is a number of caveats associated with the wide and largely uncontrolled provision of such technology during the very early phase of an epidemic. In this phase public health authorities around the world have to monitor the development of case statistics in order to make projections and attain epidemic risk assessment. The notification of false positive laboratory results can be highly detrimental during this phase of the epidemic.

The authors of this paper will provide in-vitro transcribed RNA controls to health professionals (refer to Acknowledgements section) but will not be able to provide intense technical advice. Authors will follow the policy of providing only one control, namely that for the upE assay, in order to minimise opportunities for accidental laboratory contamination. If laboratories find patient samples positive by the upE assay and control, they can conduct confirmatory testing using the ORF1b assay. A positive result in this test would most likely not be due to contamination. Of note, the target gene of our ORF1b assay does not overlap with that of other, so-called 'pan-CoV' assays [3-5], excluding the possibility of contaminating our assay with high-titred controls or PCR products from these assays.

In this light we should mention that we have been working on an N gene-based assay as well, but our experience with testing clinical material strongly suggests N-gene assays should not be used for diagnostic application for the time being, i.e., as long as no direct sequence information of the N gene is available from clinical samples.


## Acknowledgements

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## *Erratum:

Table 1 was corrected and replaced on 28 September 2012.

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# Novel coronavirus associated with severe respiratory disease: Case definition and public health measures

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**Two cases of rapidly progressive acute respiratory infection in adults associated with a novel coronavirus have generated an international public health response. The two infections were acquired three months apart, probably in Saudi Arabia and Qatar. An interim case definition has been elaborated and was published on the World Health Organization website on 25 September 2012.**

## Case 1

On 13 June 2012 a patient in their sixties presented with deteriorating pneumonia in Jeddah, Saudi Arabia and a seven day history of respiratory symptoms. The patient developed acute renal failure and died on 24 June 2012. A novel beta-coronavirus was isolated in Saudi Arabia* and sequenced at the Erasmus Medical Centre (EMC) in Rotterdam, the Netherlands [1].

## Case 2

On 11 September 2012 a patient in their forties with severe respiratory symptoms was evacuated from Qatar to a United Kingdom hospital and was admitted to intensive care there on 12 September. The patient remains in hospital and has been on life support with pulmonary and renal failure. Extensive diagnostic tests for a causative agent were negative but on 21 September a pan-coronavirus RT-PCR test performed on lower respiratory samples was positive for a conserved sequence of the coronavirus polymerase gene [2]. Comparison with the nucleotide sequence at the EMC indicated a close match with the novel virus isolated from Case 1. Contacts of Case 2, many of them healthcare workers, are being actively identified, monitored and investigated for coronavirus infection. Some of them have reported mild respiratory symptoms but none have tested positive for the novel virus or developed severe disease to date [3].

## Background

Coronaviruses are globally distributed and are found in humans, other mammals and birds. They are enveloped RNA viruses classified in alpha, beta and gamma genera. Up to one third of mild upper respiratory tract infections in adults are caused by human coronaviruses. The zoonotic severe acute respiratory syndrome (SARS) beta-coronavirus (SARS-CoV) caused the SARS outbreak in 2003 when over 900 people died. [4] Human coronaviruses are transmitted through direct contact with secretions and via aerosol droplets. Infected patients also excrete virus in faeces and urine and under certain circumstances, airborne transmission can occur from aerosolised respiratory secretions and faecal material [5].

The detection of a novel coronavirus associated with severe respiratory disease and renal failure requires urgent assessment and careful management. The United Kingdom Health Protection Agency (HPA) alerted European Union (EU) Member States and other countries via the Early Warning and Response System (EWRS) and International Health Regulations (IHR) mechanisms.

## Control measures

The HPA has recommended stringent control measures and developed an early case definition [6]. The European Centre for Disease Prevention and Control (ECDC) has developed a risk assessment in response to the cases [2]. A surveillance strategy has been agreed between ECDC and WHO with the first priority being to determine whether there are additional severe cases. The initial virology results and the separation in time of the only two confirmed cases suggest an infection that quite probably is of zoonotic origin and different in behaviour from SARS [5]. It is essential to rule out there being additional severe undiagnosed cases, especially since the transfer of severely ill patients in air ambulances meant that cases may be missed by conventional surveillance that is based on clinical notification by the original diagnosing physician, particularly primary care physicians. Hence the interim case definition has been developed with the aim of providing a high level of sensitivity for identifying cases ill

enough to require hospital care or having pneumonia while avoiding cases with only mild symptoms [7].

## Case definition

The case definition applies the established link that both cases stayed in the Arabian Peninsula but makes it conditional of hospitalisation or pneumonia, which means that cases with a link to an affected area but only mild symptoms do not require investigation. The affected area is currently defined as Saudi Arabia and Qatar but can be expanded as needed. Human coronaviruses have a short incubation period of 3 to 4 days. The longest incubation period observed during the SARS outbreak was 12 days. However, this was an outlier and a pragmatic incubation period of up to 10 days has been adopted for the case definition. The case definition should be used by clinicians for deciding which patients require investigation for possible novel coronavirus infection and which patients should be reported to national authorities. An interim case definition was published on the WHO website on 25 September [8]. It is expected to be amended once more epidemiological and diagnostic information becomes available and clinicians and public health managers should stay updated with the latest version on the website.

EU Member States have been requested to report patients meeting the case definition to ECDC through the EWRS and countries should continue to report probable or confirmed cases through the IHR contacts at WHO regional offices as mandated by the IHR. There is currently no rapid diagnostic test that easily confirms infection with this novel virus. Virus detection and serological testing is being developed by the HPA, the EMC and the University of Bonn, Germany and this was facilitated through close collaboration including the provision of preliminary sequences and a virus isolate between those institutions [9].

## Infection control advice

The HPA has developed specific infection control advice for suspected or confirmed novel coronavirus cases. The guidelines take a strict precautionary approach, whereby patients are isolated in negative-pressure single rooms or, if this is not possible then a single room with en-suite facilities. Full personal protective equipment (PPE), including gowns, gloves and FFP3 masks are worn by staff and others having direct contact with the patient [6].

## Conclusions

This situation is still evolving and there are many unknowns to consider in hypothesis generation and control measures. There is strong evidence that a novel virus caused the severe disease in the two patients. Based on this assumption it can be concluded that the virus poses an as yet poorly defined level of threat to people's health. There may have been other cases in the past that were missed and serological testing of stored sera and other specimens from such cases will be important. Serological testing will also determine whether the two cases represent the most severe end of a spectrum of clinical presentations which also includes mild and asymptomatic infections or if they are isolated events. To date, the long period between occurrence of the two cases and the lack of secondary cases among contacts suggest the disease is poorly communicable in humans. Our assessment, based on the limited information currently available, is that the risk of wide spread transmission resulting in severe disease is low. However, the emergence of a novel coronavirus requires a thorough assessment which is currently being coordinated at international level.


### The ECDC internal response team

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### *Authors' correction:

The country in which the virus was isolated was added on 28 September 2012 at the request of the authors.

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# I-MOVE: a European network to measure the effectiveness of influenza vaccines

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**Since 2007, the European Centre for Disease Prevention and Control (ECDC) has supported I-MOVE (influenza monitoring vaccine effectiveness), a network to monitor seasonal and pandemic influenza vaccine effectiveness (IVE) in the European Union (EU) and European Economic Area (EEA). To set up I-MOVE, we conducted a literature review and a survey on methods used in the EU/EEA to measure IVE and held expert consultations to guide the development of generic protocols to estimate IVE in the EU/EEA. On the basis of these protocols, from the 2008/09 season, I-MOVE teams have conducted multicentre case–control, cohort and screening method studies, undertaken within existing sentinel influenza surveillance systems. The estimates obtained include effectiveness against medically attended laboratory-confirmed influenza and are adjusted for the main confounding factors described in the literature. I-MOVE studies are methodologically sound and feasible: the availability of various study designs, settings and outcomes provides complementary evidence, facilitating the interpretation of the results. The IVE estimates have been useful in helping to guide influenza vaccine policy at national and European level. I-MOVE is a unique platform for exchanging views on methods to estimate IVE. The scientific knowledge and experience in practical, managerial and logistic issues can be adapted to monitor surveillance of the effectiveness of other vaccines.**

Human influenza viruses are subject to frequent antigenic changes. For this reason, the influenza vaccine is the only vaccine reformulated each year to optimise antigenic match between the vaccine and circulating virus strains. The seasonal influenza vaccine is a trivalent vaccine, which currently includes strains of the A subtypes H3N2 and H1N1 and one strain of B virus [1]. The World Health Organization (WHO) issues recommendations in February for which strains should be included in the seasonal vaccine for the northern hemisphere. Once these recommendations have been made, vaccine producers need at least six months to manufacture and distribute the seasonal vaccine. In a pandemic situation, pandemic strain-specific vaccines become available four to six months after the beginning of the vaccine development. During the 2009 pandemic, the influenza A(H1N1)pdm09 strain was identified in April 2009 but the first pandemic vaccines started to become available in Europe only at the end of September 2009. Consequently, antigenic changes in circulating viruses may occur before the start of the vaccination campaigns and can result in a poor match between vaccine (seasonal and pandemic) and circulating strains.

In Europe, seasonal influenza viruses circulate in the cold months, generally between October and April. National influenza surveillance networks have been established since the 1950s based on sentinel practitioner networks. In 1995, the European Influenza Surveillance Scheme was established [2]. Since 2008, the European Centre for Disease Prevention and Control (ECDC) has coordinated the European Influenza Surveillance Network (EISN) [3]. Sentinel practitioners include general practitioners (GPs), paediatricians or other physicians, depending on the European Union (EU) Member State.

Influenza vaccination is the most effective preventive measure available against influenza infection. In May 2003, the World Health Assembly recommended vaccination for all people at high risk, defined as the elderly and persons with underlying diseases. WHO Member States committed to attain a vaccination coverage in the elderly population of at least 50% by 2006 and 75% by 2010 [4]. In December 2009, the Council of the EU issued a recommendation encouraging EU Member States to take action to reach the target of 75% vaccine coverage of the older age groups recommended by WHO and if possible of other risk groups, preferably by 2014–2015 [5].

Influenza vaccination campaigns are conducted every year in the EU Member States, targeting a high number of individuals [6]. As with any public health intervention, it is important to evaluate their effectiveness. The existence of robust systems to monitor the safety and

effectiveness of vaccines is a major determinant of the success of vaccination programmes. Because of antigenic drift, vaccine effectiveness cannot be inferred from estimates from previous seasons. In order to evaluate influenza vaccine effectiveness (IVE) in Europe, ECDC developed a network to monitor seasonal and pandemic IVE in the EU and European Economic Area (EEA). In this article, we describe the phases undertaken to establish the network, its organisation and the main lessons learnt in the first four influenza seasons.

## I-MOVE preparatory phase

In 2007, under the ECDC umbrella, the network – composed initially of 18 European public health institutes and EpiConcept, the coordinating hub – was set up. Named I-MOVE (influenza monitoring vaccine effectiveness) in Europe, it aimed to measure IVE on a routine basis. The study methods had to be simple, sustainable in a context of limited budget, adapted to the EU/EEA context and scientifically robust.

The objectives of the first preparatory phase of I-MOVE were to identify the most appropriate observational study designs to measure IVE routinely in the EU/EEA and to identify key methodological issues to be considered in the study protocols. To achieve these objectives, EpiConcept conducted the following: (i) a survey among EU/EEA Member States to identify IVE studies performed in Europe and potential data sources for future studies; (ii) a literature review on methods used to estimate IVE; and (iii) three expert consultations. The methods and results of this phase have been described elsewhere [7]. In brief, the main conclusions were that EU/EEA influenza sentinel surveillance systems seemed to provide a sustainable platform suitable for case–control studies and screening method studies monitoring IVE. In Member States or regions with computerised primary care databases, cohort studies could be conducted to measure IVE against different outcomes.

To control for positive and negative confounding, a minimum set of variables had to be collected in all studies [8]. In addition, as using a specific outcome reduces bias, it was recommended to measure IVE against laboratory-confirmed influenza.. To minimise selection bias, sentinel practitioners were to select systematically the patients to swab.

On the basis of these conclusions, EpiConcept and ECDC developed and published generic case–control, cohort, screening method and cluster investigation protocols for IVE studies [9-11] to be adapted by each potential study site. An expert panel selected seven protocols for the 2008/09 pilot season, and eleven for the2009/10 (pandemic season), 2010/11 and 2011/12 seasons (Table 1).

## I-MOVE organisational aspects

In the first four seasons, 26 partner institutions from 17 EU/EEA Member States participated in I-MOVE (Figure 1). Each institution designated an I-MOVE focal point, most of them being influenza experts coordinating the national influenza surveillance system. A total of 13 study site teams have conducted IVE studies: some use several study designs in the same site during the same season (Table 1). The functioning of the I-MOVE network has to date been funded by ECDC and the IVE studies co-funded by ECDC and the study sites. EpiConcept coordinates the I-MOVE activities. The network collaborates with teams conducting IVE studies in Canada, USA and Australia.

Technical workshops are organised during the influenza season among I-MOVE study sites to discuss the preliminary results, to plan the final analysis and to define the publication strategy. Periodically, follow-up videoconferences are organised between study sites. The whole network meets annually at the end of the influenza season to share the IVE estimates and discuss practical and methodological issues related to the studies. Since the 2009/10 season, the last day of the meeting has been open to decision-makers (European Medicines Agency (EMA), European Commission, WHO) and vaccine manufacturers. An I-MOVE website is in place with three different levels of access: unrestricted, restricted to I-MOVE partner institutions, restricted to I-MOVE study sites [12].

Each study site defines its strategy to communicate its results. EpiConcept, in close collaboration with ECDC, coordinates the publication of the multicentre case–control pooled results. Since 2008, I-MOVE IVE results have been published in peer-reviewed journals [13-32].

## I-MOVE implementation phase (2008/9 to 2011/12)

The study designs used within I-MOVE are case–control studies, cohort studies using primary care databases and screening method studies.

Case–control studies, including multicentre case–control study

Nine EU sites have contributed to the multicentre case–control study (Table 1). The methods used for the individual and multicentre case–control studies have been described elsewhere [21,22,25,27-30,32]. In summary, each season the study site coordinators invite sentinel primary care practitioners belonging to the national sentinel surveillance systems to participate in the study. In Portugal, Italy, and Hungary, practitioners other than those participating in the sentinel surveillance system have been also invited to participate.

The study population in each case–control study consists of non-institutionalised patients consulting a participating practitioner for ILI or ARI (France) within eight days after symptom onset. The age groups and covariates included in the study have varied from one season to another (Table 2). Practitioners take nasal or throat swabs from all or a sample of ILI/ARI patients. From the

**Table 1**

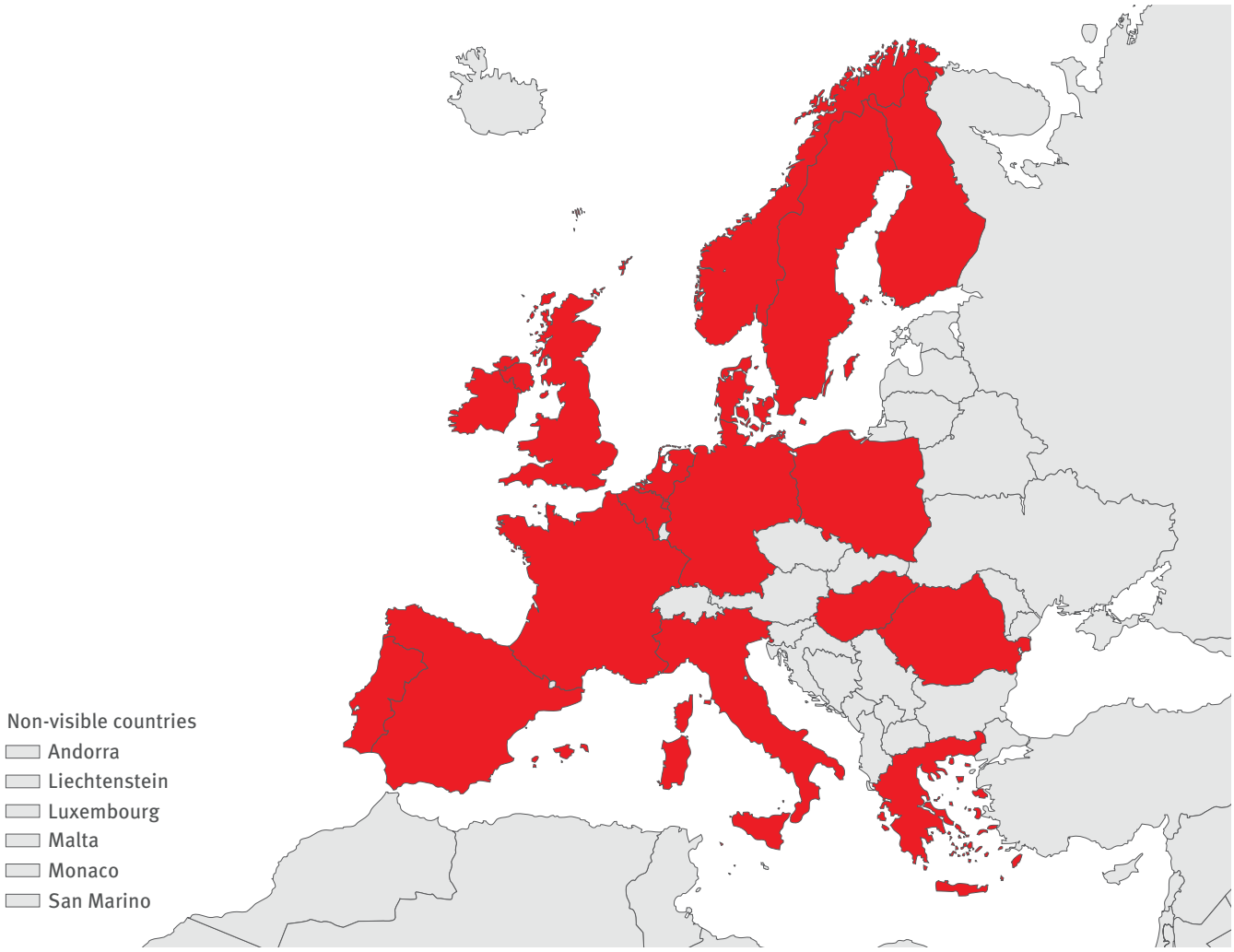
Sites conducting influenza vaccine effectiveness studies as part of I-MOVE, influenza seasons 2008/09 to 2011/12

| Country and institution/network | Influenza season | Case–control studies based on primary care sentinel networks | Cohort studies using primary care computerised databases, including nested case–control studies | Screening method studies | Multicentre case–control study |
|---|---|---|---|---|---|
| Denmark, Statens Serum Institut | 2008/09 | x | – | – | x |
| England and Wales, Royal College of General Practitioners | 2009/10 | – | x | – | – |
| | 2010/11 | – | x | x | – |
| | 2011/12 | – | x | x | – |
| France, Réseau des GROG (Groupes Régionaux d'Observation de la Grippe) | 2009/10 | x | – | – | x |
| | 2010/11 | x | – | – | x |
| | 2011/12 | x | – | – | x |
| Hungary, Office of the Chief Medical Officer and National Centre for Epidemiology | 2008/09 | x | – | – | x |
| | 2009/10 | x | – | – | x |
| | 2010/11 | x | – | – | x |
| | 2011/12 | x | – | – | x |
| Ireland, Health Protection Surveillance | 2009/10 | x | – | – | x |
| | 2010/11 | x | – | – | x |
| | 2011/12 | x | – | – | x |
| Italy, Istituto Superiore di Sanità | 2009/10 | x | – | x | x |
| | 2010/11 | x | – | x | x |
| | 2011/12 | x | – | x | x |
| Poland, National Institute of Public Health – National Institute of Hygiene | 2010/11 | x | – | | x |
| | 2011/12 | x | – | – | x |
| Navarre (Spain), Instituto de Salud Pública de Navarra | 2008/09 | – | x | – | – |
| | 2009/10 | – | x | – | – |
| | 2010/11 | – | x | – | – |
| | 2011/12 | – | x | – | – |
| Netherlands, Erasmus University | 2009/10 | – | x | – | – |
| Portugal, Instituto Nacional de Saúde Dr Ricardo Jorge | 2008/09 | x | – | x | x |
| | 2009/10 | x | – | x | x |
| | 2010/11 | x | – | x | x |
| | 2011/12 | x | – | x | x |
| Romania, Cantacuzino Institute, National Center for Research and Development in Microbiology and Immunology | 2008/09 | x | – | – | x |
| | 2009/10 | x | – | – | x |
| | 2010/11 | x | – | – | x |
| | 2011/12 | x | – | – | x |
| Scotland, Health Protection Scotland | 2009/10 | – | x | – | – |
| | 2010/11 | – | x | x | – |
| | 2011/12 | – | x | x | – |
| Spain, Instituto de Salud Carlos III | 2008/09 | x | – | x | x |
| | 2009/10 | x | – | x | x |
| | 2010/11 | x | – | x | x |
| | 2011/12 | x | – | x | x |
| United Kingdom study including Health Protection Scotland and the Royal College of General Practitioners | 2008/09 | – | x | – | – |

I-MOVE: Influenza monitoring vaccine effectiveness.
x indicates that the study was carried out.

**Figure 1**

European Union and European Economic Area Member States with I-MOVE partner institutions, influenza seasons 2007/08 to 2011/12




I-MOVE: Influenza monitoring vaccine effectiveness.

Countries in red are Member States with I-MOVE partner institutions.

third season, all study sites selected systematically ILI/ARI patients to swab.

Study sites have progressively adopted the EU ILI case definition [33]: four sites in 2008/09 and seven from 2009/10.

We defined a case of influenza as an ILI patient who tests positive for influenza using reverse transcription polymerase chain reaction (RT-PCR) or culture. Controls are ILI patients testing negative for influenza (test-negative controls). Depending on the study site, testing is performed at national or regional reference laboratories. All laboratories testing sentinel specimens within the EISN scheme are part of a community network of reference laboratories (CNRL), which undergo periodic external quality assessments for virus detection and characterisation methods [34].

The sentinel practitioners interview the ILI/ARI patients face-to-face, collect information on a set of predefined variables common to all study sites (Table 1) and send the completed questionnaires to each of the I-MOVE study site coordinators.

National study teams send to the EpiConcept coordination team anonymised databases of recruited ILI cases. We evaluate heterogeneity between studies qualitatively and quantitatively [35,36]. We estimate the pooled IVE using a one-stage method, with the study site included as fixed effect in the model. To estimate adjusted IVE, we use a logistic regression model

## Table 2

Characteristics of I-MOVE multicentre case–control study, influenza seasons 2008/09 (5 study sites), 2009/10 (7 study sites), 2010/11 (8 study sites), 2011/12 (8 study sites)

| Item | 2008/09 | 2009/10 | 2010/11 and 2011/12 |
|---|---|---|---|
| Participating countries/study sites | DK, PT, ES, RO, HU | PT, ES, RO, HU, IT, IE, FR | PT, ES, RO, HU, IT, IE, FR, PO |
| Study population (restricted to non-institutionalised patients) | Aged ≥65 years, 4 study sites<br>HU: ›59 years | All ages, 6 study sites<br>HU: ›17 years<br>RO: ›15 yrs<br>IT: target population for vaccination | All ages, 7 study sites<br>HU: ›17 years |
| Influenza-like Illness case definition | EU case definition[a], 4 study sites<br>PT: GP clinical criteria | EU case definition[a], 7 study sites<br>IT: WHO case definition[b] | EU case definition[a], 7 study sites[b] |
| Patients selected for swabbing | | | |
| Elderly | All | All | All (not in Italy in 2010/11)[c] |
| Other age groups | Not included | Systematic sampling, 7 study sites<br>IE: 5 ILI cases/GP/week | Systematic sampling |
| Information on co-variables collected | | | |
| Age | Yes | Yes | Yes |
| Sex | Yes | Yes | Yes |
| Symptoms | Yes | Yes | Yes |
| Date of symptom onset | Yes | Yes | Yes |
| Date of swabbing | Yes | Yes | Yes |
| Presence of chronic diseases | Yes | Yes | Yes |
| Hospitalisations for chronic disease in previous 12 months | Yes[d] | Yes | Yes |
| Smoking history | Yes | Yes | Yes (not in France in 2011/12) |
| Functional status | Yes | Yes | Yes (not in Spain in 2011/12) |
| Influenza vaccination in previous season | Yes | Yes | Yes |
| Influenza vaccination in current season | Yes | Yes | Yes |
| Date of vaccination in current season | Yes | Yes | Yes |
| Vaccine brand | No | Yes | Yes |
| Number of practitioner visits in previous season | No | Ye | Yes |
| Pregnancy | No | Yes | Yes |
| Obesity[e] | No | Yes | Yes[f] |
| Belonging to target population for vaccination | No | No | 5 study sites in 2010/11[g]<br>7 study sites in 2011/12[g] |

DK: Denmark; ES: Spain; FR: France; HU: Hungary; IE: Ireland; IT: Italy; PO: Poland; PT: Portugal; RO: Romania.

EU: European Union; GP: general practitioner; ILI: Influenza-like illness: I-MOVE: influenza monitoring vaccine effectiveness; WHO: World Health Organization.

[a] EU ILI definition: sudden onset of symptoms and at least one of the following four systemic symptoms: fever or feverishness, malaise, headache, myalgia and at least one of the following three respiratory symptoms: cough, sore throat, shortness of breath [33].

[b] ILI case definition used in Italy: sudden onset of fever, temperature ›38 °C and cough or sore throat in the absence of another diagnosis

[c] Italy: one person aged ›64 years swabbed per week in 2010/11.

[d] Hungary and Portugal: any hospitalisation in previous 12 months.

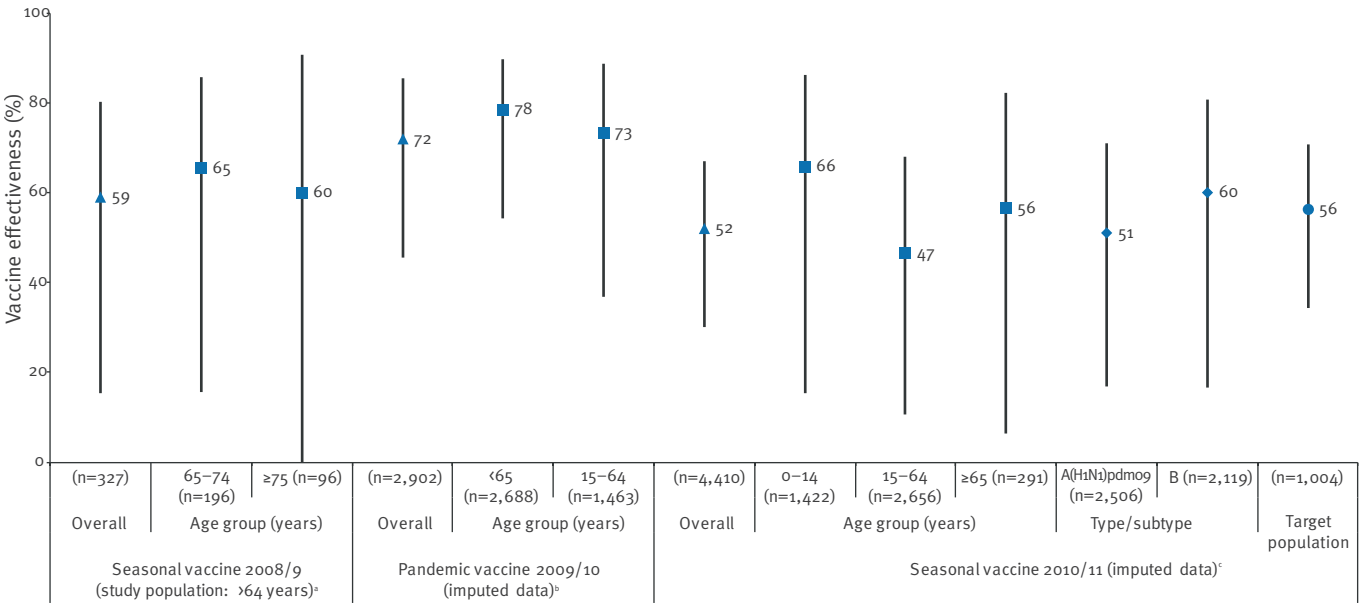
[e] Obesity defined based on body mass index (≥30 in FR, IT, PO, PT; ≥35 in HU; ≥40 IE, ES); defined as "Obesity Yes/No/Unknown" in RO.

[f] Information on obesity not collected in France and Poland in 2010/11.

[g] Information on whether patients belonged to target population not collected in 2010/11 in France, Hungary and Italy; not collected in France in 2011/12.

**Figure 2**

Adjusted overall and stratified influenza vaccine effectiveness against medically attended laboratory-confirmed influenza, I-MOVE multicentre case–control study, 2008/09 (5 study sites), 2009/10 (7 study sites), 2010/11 (8 study sites)




I-MOVE: Influenza monitoring vaccine effectiveness.

[a] Adjusted for previous season influenza vaccination, at least one chronic disease, sex, at least one hospitalisation in previous 12 months, current smoker, age group (not included in the age-group strata), functional status.

[b] Adjusted for any influenza vaccination in the two previous seasons, 2009/10 seasonal influenza vaccination, at least one chronic disease, sex, at least one hospitalisation for chronic disease in previous 12 months, current smoker, age group, practitioners vists in previous 12 months, month of symptom onset.

[c] Adjusted for influenza vaccination in previous 2 seasons, at least one chronic disease, sex, at least one hospitalisation for chronic disease in previous 12 months, current smoker, age group, practitioners visits in previous 12 months, week of symptom onset.

including all potential confounding factors. In 2009/10 and 2010/11 seasons, we estimated missing data for vaccination status and covariates using the multiple multivariate imputation by chained equations procedure in STATA [37].

The number of participating primary care practitioners/practices was 343 in 2008/09, 1,114 in 2009/10, 1,035 in 2010/11, 942 in 2010/11, and 1,056 in 2011/12. The sample size increased in the first three seasons: in 2008/09, the pilot season, the study was restricted to individuals aged 65 years or more and 327 ILI cases were included in the pooled analysis. In 2009/10, 2010/11 and 2011/12, the number of ILI patients recruited were 2,902, 4,410 and 4,747, respectively.

All cases included in the 2009/10 study were laboratory confirmed as influenza A(H1N1)pdm09. Therefore, the effectiveness of the monovalent pandemic vaccine (72%; 95% CI: 48 to 85) was pandemic strain specific. Estimates of the trivalent 2010/11 seasonal vaccine effectiveness were lower than the pandemic IVE: 52% (95% CI: 30 to 67) overall, 51% (95% CI: 17 to 71) against influenza A(H1N1)pdm09 and 56% (95% CI: 34 to 71) when restricting the analysis to the target group for vaccination (Figure 2). In the age group 15–54 years, the point estimate for the pandemic vaccine effectiveness (73%) was higher than the point estimate for the effectiveness of the trivalent seasonal vaccines in 2009/10 (65%) and 2010/11 (47%). In the 2011/12 season, preliminary results (April 2012) suggested an overall low adjusted effectiveness (27 %) against influenza A(H3N2) among persons targeted for vaccination [24]. In Spain, early (25 December 2011 to 19 February 2012) IVE in the target population was 54% [19].

## Cohort studies

Four study sites have conducted cohort studies (Table 1). These studies are based on electronic primary care databases that, using a unique identifier, can be linked

## Table 3

Variables collected, data sources and timing of data extraction in I-MOVE cohort studies in Navarre (Spain), Royal College of General Practitioners (England and Wales) and Scotland, influenza season 2011/12

| Type of variable | Variables | Data source | Timing of data extraction | | |
|---|---|---|---|---|---|
| | | | Navarre, Spain | Scotland | England |
| Demographic characteristics | Age, date of birth, sex, location | Primary care records | Beginning of season | Beginning of season | Beginning of season |
| Exposure: influenza vaccination for the study season | Vaccination and date | Primary care records | Weekly | Daily | Twice weekly |
| | Type of vaccine | Primary care records | Weekly | Not available | Not available |
| Outcomes | Medically attended Influenza-like Illness | Primary care records | Daily | Daily | Twice weekly |
| | Upper respiratory tract infections | Primary care records | Not available | Not available | Twice weekly |
| | Acute respiratory infections | Primary care records | Daily | Daily | Twice weekly |
| | Hospitalisations for influenza or pneumonia | Hospital discharge | End of season | Available at end of season (not used until now) | Not available |
| | Death | Primary care records | Weekly | Daily | Twice weekly |
| | Severe acute respiratory infections | Hospital discharge | Daily | Not available | Not available |
| | Medically attended laboratory-confirmed influenza | Laboratory reports | Daily | Every five days | Twice weekly |
| Confounding factors | Underlying Chronic diseases | Primary care records | Beginning of season | Beginning of season (daily) update) | Beginning of season |
| | Primary care visits in previous year (for Scotland: influenza-like illness, acute respiratory infections visits) | Primary care records | Beginning of season | Beginning of season | Beginning of season |
| | Hospitalisations for influenza or pneumonia in previous season | Hospital discharge database | Beginning of season | Not available | Twice weekly |
| | Number of prescriptions in previous year | Primary care records | Not available | Beginning of season | Beginning of season |
| | Index of multiple deprivation, based on patient´s postcode | Primary care records | Not available | Beginning of season | Beginning of season |
| | Number of antibiotic prescriptions in previous year | Primary care records | Beginning of season | Beginning of season | Twice weekly |
| | Pneumococcal vaccination and date | Primary care records | Beginning of season for past years; weekly for current season | Beginning of season for past years; weekly for current season | Beginning of season for past years; twice weekly for current season |
| | Influenza vaccination in previous seasons | Primary care records | Beginning of season | Beginning of season | Beginning of season |

I-MOVE: influenza monitoring vaccine effectiveness.

to other databases such as a vaccination registry, hospital admissions or laboratory databases. The linkage of the databases provides information on exposure, various outcomes and potential confounding factors or effect modifiers (Table 3). Consequently, using a person-time analysis, cohort studies estimate adjusted IVE against various clinical outcomes (ILI, ARI, lower respiratory tract infection, hospital admission and death). In the 2010/11 season, the size of the cohorts varied from 93,380 individuals in Scotland to 1,005,132 in England.

Within the cohort studies, nested test-negative case–control studies are conducted to estimate IVE against medically attended laboratory-confirmed influenza [14,16,17,20,26]. During the 2009/10 season, the cohort in Scotland gave an estimated adjusted IVE of 49% (95% CI: 19 to 67) for ILI, of 40% (95% CI: 18 to 56) for overall mortality and of 60% (95% CI: –38 to 89) for virologically confirmed symptomatic individuals [20]. During the same season, the cohort study in England and Wales estimated an adjusted IVE of 21% (95% CI: 5.3 to 34.0) in preventing ILI and of 64% (95% CI: –6 to 88.6) in preventing PCR-confirmed influenza A(H1N1)pdm09 [18]. The Navarra cohort (Spain) results were similar: vaccination with the 2009 pandemic vaccine was associated with an adjusted 32% (95% CI: 8 to 50) reduction in the overall incidence of medically attended ILI and an adjusted 89% (95% CI: 36 to 100) reduction in the incidence of PCR-confirmed influenza [15].

## Screening method studies

In addition to estimating IVE using a cohort or a case–control study, some study sites use the screening method (Table 1). In the screening method, IVE is estimated by comparing the vaccination coverage between ILI patients positive for influenza and a reference group. The reference groups used in I-MOVE studies vary by study site and include the vaccination coverage in the practitioners´ catchment area (e.g. Spain, Scotland, England), the vaccination coverage in a random sample of the population (Portugal [25]) or in the general population (Italy [31]). The Farrington method [38] is used to adjust IVE for age group (Spain, Italy, Portugal, Scotland), risk group (Portugal, Scotland), GP practice (Scotland) and socio-economic status (Scotland). During the pandemic and in line with results using other study designs, the I-MOVE screening method studies estimated a high pandemic IVE against medically attended laboratory-confirmed influenza A(H1N1)pdm09: the IVE was 78% (95% CI: 61 to 88) in the Spanish study [39] and 92% (95% CI: 46 to 99) in the Italian [31].

# Conclusion

The I-MOVE network is well established and has provided seasonal and pandemic IVE for four consecutive influenza seasons (2011/12 results have been submitted for publication). The I-MOVE results are timely: since the 2009/10 season, preliminary results have been communicated early in the season to the decision-makers and published in peer-reviewed journals [15,19,23,24,29].

I-MOVE results have assisted in guiding public vaccination policy at national and European level. In particular, during the 2009 pandemic they contributed to the risk–benefit analysis process coordinated in the EU by the EMA [40] and globally by WHO [41] by providing regular updates of IVE estimates. In 2012, the low observed IVE against influenza A(H3N2) prompted a discussion on the respective role of antigenic drift and early waning immunity [19,24]. In addition, the European regulatory authority (EMA) incorporated I-MOVE estimates as a component of post-licensure surveillance for the 2009 pandemic vaccines [42]. As the I-MOVE IVE studies are conducted by an independent scientific research network, this adds weight to the integrity of their results and to how they are perceived professionally and by the public.

Using a sound methodology, I-MOVE studies have shown that seasonal IVE is moderate against medically attended laboratory-confirmed influenza. This is triggering a number of initiatives including a possible revision of EU regulatory criteria for annual vaccine relicensure that include results of IVE studies [43]. Given the timely provision of in-season estimates of IVE from I-MOVE and similar networks elsewhere in the world, discussions are ongoing with WHO to consider how such estimates can contribute to the annual vaccine strain selection process [44].

During the 2009/10 pandemic season, all the I-MOVE network participants (practitioners, laboratories, national and regional public health institutes) were overwhelmed with response activities. Having the I-MOVE coordinating hub based in a structure not directly involved in the response was an advantage: the studies were not disrupted and the coordination hub could focus on facilitating exchanges between study sites, on rapidly analysing the multicentre case–control study and on coordinating the communication of IVE results to ECDC.

I-MOVE is a unique platform for exchanging views on and experience of methods to estimate IVE. During the I-MOVE technical workshops and annual meetings, the discussions around the epidemiological and logistic challenges allowed improvement of standard methods, good scientific practices to be followed and have strengthened EU expertise on IVE. The network has contributed to strengthening influenza surveillance in the EU. Currently, most of the sentinel practitioners conducting I-MOVE studies use the same EU ILI case definition and select patients for swabbing in a systematic way. As most I-MOVE practitioners are part of the national sentinel surveillance systems, any improvement and standardisation of methods should have a positive impact on national and European influenza surveillance systems.

The I-MOVE network takes into account the operational and methodological aspects required to building a sustainable system: studies are methodologically sound but at the same time feasible within existing surveillance systems and with limited resources. The estimates include effectiveness against medically attended laboratory-confirmed influenza as outcome and are adjusted for the main confounding factors described in the literature (e.g. presence of underlying chronic diseases, health-seeking behaviour, age group, etc.). In addition, the availability of various study designs, settings and outcomes gives a combination of different sets of evidence, facilitating better interpretation of the results.

The challenges for monitoring IVE in Europe include the variety of influenza vaccines available and in use and differences in vaccination coverage and groups targeted for vaccination and in health-seeking behaviours between Member States [45]. Having estimates by vaccine type and among target groups represents a major challenge and requires large sample sizes. The constant increase in sample size observed throughout the four influenza seasons and the precise information collected on vaccine type and brand could allow estimation of IVE by vaccine type in the near future. Time, especially during a pandemic, needs to be accounted for in the analysis (e.g. adjustment for week/month of symptom onset, person-time analysis in the cohort studies). The main limitation in reaching a large sample size is the low influenza vaccination coverage in some groups [46]. Pooling data from the various I-MOVE case–control studies is one of the I-MOVE strengths that allows IVE to be estimated early in the season and for different subgroups. In 2010/11, we had for the first time IVE estimates for the target groups for vaccination [22]. However, results still lack sufficient precision and efforts should be made to increase the sample size in each study site.

In I-MOVE, the cohort study in the Navarre region of Spain is the only study able to provide early and repeated estimates of IVE against hospital admission of persons with laboratory-confirmed influenza [16]. Therefore, one of the limitations of I-MOVE is that it does not yet provide early estimates of IVE against severe outcomes at European level. The main challenge is to attain a sufficient sample size enabling precise adjusted estimates and stratification by effect modifiers. A European hospital network with multiple sites using the same protocol would allow a multicentre study to be conducted with a sample size sufficient to rapidly estimate IVE against severe influenza outcomes. As a first step, the I-MOVE network has developed a generic protocol for IVE hospital case–control studies. From the 2011/12 season, hospitals in the Valencia and Navarre regions (Spain), France and Italy are conducting studies based on this protocol and are providing pooled estimates of IVE against hospital admissions with laboratory-confirmed influenza.

Some countries considered that the screening method, in addition to case–control and cohort studies, was convenient because of its inexpensiveness, reliance on already available data and ability to provide an early indication of IVE.

Influenza sentinel surveillance networks have shown to be an excellent framework in which IVE observational studies can be conducted using different study designs (cohort, case–control and screening method) not only in Europe but also in other countries such as Canada or Australia [47,48]. The scientific knowledge and experience in practical, managerial and logistic issues gained by the I-MOVE network can be used in other regions of the world to estimate IVE. The I-MOVE model can also be adapted to establish similar monitoring systems in Europe for vaccines that may change their effectiveness over time due to, for example, serotype replacement or to changes in vaccination schedules (e.g. rotavirus, pneumococcal conjugate vaccine). I-MOVE experience, protocols and some of the study sites infrastructures are already contributing to a recent ECDC project for assessing vaccination impact and effectiveness studies for pneumococcal conjugate vaccine [49].

There is a strong case for use of the I-MOVE methodology for monitoring IVE as a routine part of post-licensure monitoring. The approach described in this article is ethical and practical. While it cannot have the accuracy of randomised controlled trials, the results achieve their objective in detecting changes in effectiveness over time and with changes in vaccine.

The major challenge is how to make these studies sustainable. While they are not as expensive as randomised controlled trials, they are not as inexpensive as the sentinel surveillance undertaken by the same practitioners. Nevertheless, they require accurate virus testing and careful coordination to retain quality. What has yet to be resolved is how to attract co-sponsorship from industry and public sectors while retaining independence. Some manufacturers appreciate the advantages to them of having such validation as do regulators. However, a way of combining monies in a share scheme has yet to be achieved. The recent breakthrough of agreement for sustaining WHO's essential influenza surveillance work may show the way forward [50].

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# Survey of delivery of prophylactic immunoglobulins following exposure to a measles case

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**In France, almost 23,000 cases of measles and 10 deaths have been reported between January 2008 and August 2012. French health authorities recommend delivery of human polyvalent immunoglobulins in the event of exposure to a measles case for some categories of unvaccinated persons (children under the age of 12 months, immunocompromised persons and pregnant women), within six days after exposure and following laboratory confirmation of the contact case. We carried out a postal survey among 368 French hospital pharmacies to evaluate the number of persons affected by this measure between 1 January 2010 and 31 August 2011, to describe the characteristics of these patients and to evaluate the application of the recommendations in terms of delay between exposure and immunoglobulin delivery, and confirmation of the contact case. The response rate to the survey was 73%. In total, 400 immunoglobulin deliveries were listed, most of them for children under the age of one year, and 84% of the 250 administrations with available information occurred within six days after exposure, as recommended. However, only 48% of the 209 treated contacts with available information were laboratory-confirmed when the immunoglobulins were delivered. This survey is the first evaluation of this recommendation since its introduction in 2005 and suggests that the recommendations may need to be updated.**

## Introduction

Measles is a highly contagious illness, characterised by a rash associated with a cough and fever (>38 °C). The disease is often mild, but serious complications can occur, mainly pneumonia and encephalitis, leading in some cases to death. These complications occur more frequently in children younger than one year and in adults older than 20 years [1-3].

In France, a single-dose vaccination against measles was introduced in 1983, and in 1997, recommendations increased to two doses. The current vaccination schedule is as follows: a first dose at the age of 12 months (or at nine months for children attending nurseries) and a second dose for children between the age of 13 and 24 months [4]. Catch-up immunisation for those born after 1980 was introduced in 2005 [5], and since February 2011, it has been recommended that all those born after 1980 receive two doses of combined measles-mumps-rubella (MMR) vaccine for full protection [6].

Within the framework of the measles elimination policy followed in the World Health Organization European Region since 1998 [7], a measles elimination plan was launched by the French Ministry of Health in 2005 [5]. This plan, updated by a circular in 2009 [8], reinstated measles on the list of notifiable diseases and described the preventive measures to be implemented when there was a case or a cluster of measles cases. Persons who have been in contact with a measles case and who are not vaccinated against measles should be vaccinated within 72 h of exposure. For some categories of people seen too late to be vaccinated or who cannot be vaccinated, it is recommended that they receive human polyvalent intravenous immunoglobulins after laboratory (virological) confirmation of the contact case, within six days after exposure. The categories are the following:

- unvaccinated pregnant women without a history of measles;
- immunocompromised patients, regardless of their vaccination status and measles history;
- children under the age of six months whose mother has measles;
- children under the age of six months whose mother has no history of measles and is not vaccinated;
- children between 6 and 11 months, not vaccinated against measles within 72 h of exposure, regardless of the vaccination status and measles history of the mother.

Despite the efforts towards elimination, measles outbreaks have become more frequent in France since January 2008, with the highest epidemic peak occurring in 2011 (604 cases notified in 2008, 1,544 in 2009,

5,071 in 2010, 14,966 in 2011 and 728 between January and July 2012), and have resulted in 10 deaths [9]. This situation is linked to the insufficient vaccine coverage and to the existence of communities of susceptible people in which the virus circulates actively [10,11].

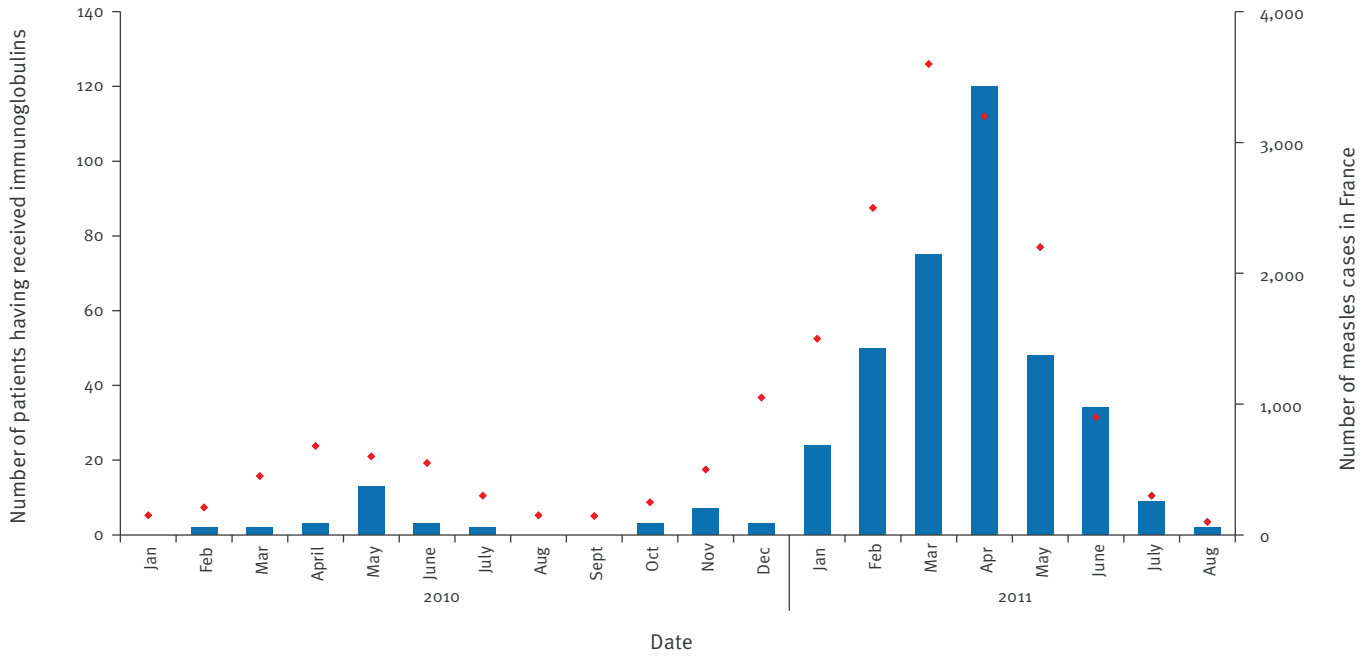
Since the implementation of the measures to fight measles in France, there have not been any field evaluations. The objectives of the present survey were to quantify the number of patients who had received prophylactic immunoglobulins after exposure to a measles case, to describe basic characteristics of these patients and to study the application of the recommendations related to the delay of post-exposure and the laboratory confirmation of the contact cases.

## Methods

The case definition of measles used in this survey was the one recommended by the World Health Organization [12]: any person in whom a clinician suspects measles infection or any person with fever and maculopapular rash and cough, coryza or conjunctivitis. Since the delivery of immunoglobulins must be done at a hospital, this survey was conducted in hospital pharmacies in metropolitan France. The French National Council of the Order of Pharmacists provided us with a file containing the names of the department heads of pharmacies in major and minor hospitals (not local ones). We added to this list further pharmacies using information from the French hospital federation (FHF, www.fhf.fr). After having excluded psychiatric hospitals, we had a list of 368 pharmacies to contact. Each pharmacy received a letter, a file explaining the aims of the study, and a questionnaire containing six questions and a table to fill out. The study targeted immunoglobulin delivery between 1 January 2010 and 31 August 2011. The following items were included in the survey: type of hospital and geographical location, delivery of prophylactic immunoglobulins after exposure to measles during the study period; if applicable, the number of doses delivered; supply difficulties for immunoglobulins during the study period; if applicable, the precise period during which supply difficulties were experienced. If they had delivered immunoglobulins, the pharmacists, assisted by physicians if necessary, provided the following information for each case: date of immunoglobulin delivery, age and sex of the patient, category of the patient (child under the age of six months, child between 6 and 11 months, pregnant woman, immunocompromised patient, other), measles vaccination status and number of doses, delay between the exposure and immunoglobulin delivery, laboratory confirmation of the contact case when the immunoglobulins were delivered, type of laboratory confirmation, and measles infection of the mother for children under the age of six months. The survey was sent by post on 26 October 2011. An email reminder was sent between 22 and 24 November 2011. The final deadline for answers was 1 December 2011. The results were analysed using Excel 2010 and R2.13.1 softwares. For quantitative variables, results are presented as mean±standard deviation.

**Figure 1**

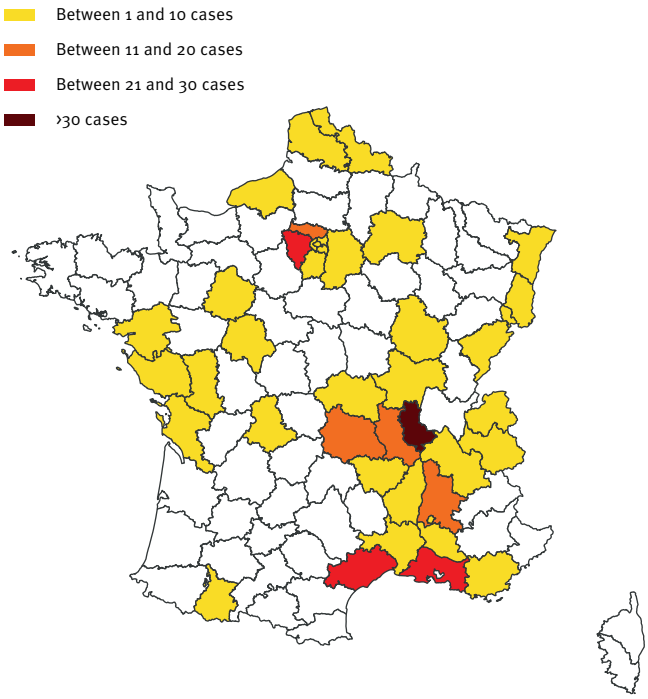
Time pattern of immunoglobulin deliveries in metropolitan France (n=400) and number of notified measles cases in France (n=19,335), 1 January 2010 to 31 August 2011




blue bars: immunoglobulin deliveries; red dots: measles cases.
Source: [13].

**Figure 2**

Geographical distribution of patients receiving prophylactic immunoglobulins after exposure to measles, metropolitan France, 1 January 2010 to 31 August 2011 (n=400)




Note: Départements in white did not deliver immunoglobulins following exposure to measles.

## Results

In total, 267 of 368 pharmacies replied to the survey, a response rate of 73%. For 13 hospitals or hospital groups, we received joint responses for several (between two and four) pharmacies linked to these structures. The 267 pharmacies therefore belonged to 246 hospitals located in 92 (of 96) geographical Départements of metropolitan France (data not shown).

Of 246 hospitals, 55 (22%) delivered prophylactic immunoglobulins after exposure to measles during the study period (1 January 2010 to 31 August 2011). Among the 55 hospitals delivering immunoglobulins, 34 were hospital centres and 21 university hospitals. In total, 400 patients received immunoglobulins. The mean number of immunoglobulins delivered per hospital was 7.3±22.4 (range: 1–166), 4±0.8 for hospital centres (range: 1–20) and 12.3±7.4 for university hospitals (range: 1–166).

Most deliveries occurred between March and May 2011 (Figure 1). This pattern is very similar to that of the measles epidemic in France, which peaked in March to April 2011.

Some geographical areas in France were more affected than others: the Paris region, the northern region, the Atlantic coast, the eastern and the south-eastern regions (Figure 2). Immunoglobulin delivery was highest in the Rhône Département with 166 doses (42%). This pattern is consistent with the geographical distribution of notified measles cases over the same time period, with a concentration of the cases in south-eastern France, in Paris and to a lesser extent along the Atlantic coast [13,14]. This geographical distribution was independent of the number of answers per Département (data not shown).

The age of the patients ranged between one day and 86 years (Figure 3). Of 400 patients, 172 were children under the age of six months, and 55 were between 6 and 12 months-old. These two age categories represented more than 55% of the cases. The third most represented age group were the 26 to 30 year-old adults, with 25 cases (6.3%). Among our patients, 24% were older than 30 years. Over the same time period, the age group for which most measles cases were notified in France were children under the age of one year (incidence: 140/100,000 cases, representing 7% of the notified measles cases) and young adults between 15 to 19 years (incidence: 60/100,000 cases, representing 17% of the cases) [13,14]. Adults older than 30 years represented 12% of the notified measles cases (incidence less than 2/100,000 cases).
)
Possible categories based on French recommendations were “children under six months-old”, “children between 6 and 11 months-old”, “pregnant women”, “immunocompromised persons” or “others” [6]. These categories were not mutually exclusive so that, for instance, a nine month-old immunocompromised child

**Figure 3**

Age distribution of patients receiving prophylactic immunoglobulins following exposure to measles, metropolitan France, 1 January 2010 to 31 August 2011 (n=400)

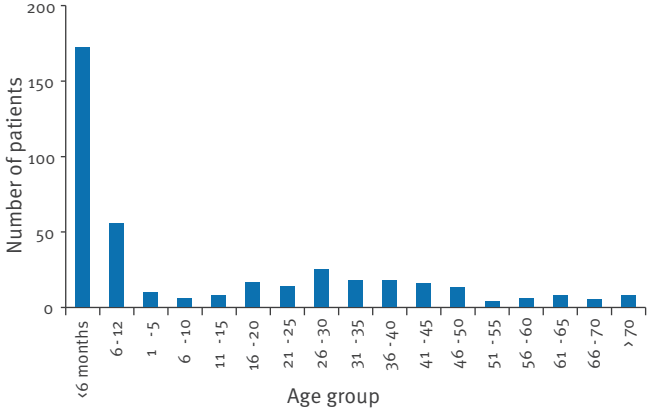
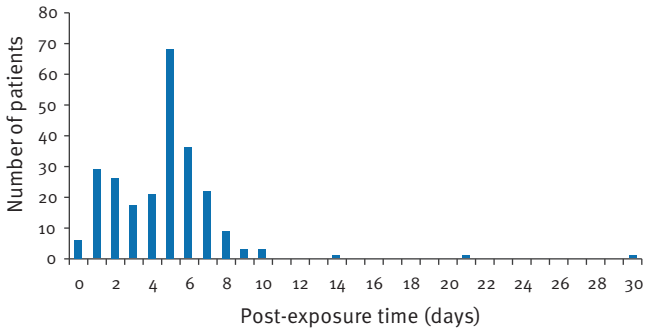


**Figure 4**

Estimated delay between the exposure to measles and the administration of prophylactic immunoglobulins, metropolitan France, 1 January 2010 to 31 August 2011 (n=243)




Note: Only precise answers are presented in this figure.

was counted in both categories "children between 6 and 11 months-old" and "immunocompromised persons". In our survey, 172 cases (42%) were children under six months-old, 55 (14%) were children between 6 and 11 months-old, 47 (12%) were pregnant women, 120 (29%) were immunocompromised persons, seven (1.7%) were others, and there were seven with missing data (1.7%). Eight patients belonged to two categories: "children under six months-old" and "immunocompromised persons".

The mean delay between exposure and immunoglobulin delivery was 4.6 days (range: 0–30 days). The median was five days. The most frequent delays were five and six days (Figure 4). There were 146 cases of missing data and 11 imprecise responses for this variable (n=157 in total, i.e. 39% of the cases). This low response rate underlines the difficulty of estimating the post-exposure delay. Among the 11 imprecise answers, seven were precise enough to be categorised either as delay of six days or less, or as delay of more than six days. Of these 250 answers, 84% of administrations (n=209) occurred within six days after exposure, as recommended. Three cases of very late immunoglobulin delivery (14, 21 and 30 days after exposure) were observed (Figure 4). In one case, the pharmacist (and/or physician) identified the rash as the beginning of the exposure (added as a comment in the answer).

Data regarding the laboratory (virological) confirmation of the contact case was also missing frequently, in 191 (48%) of the cases. In the 209 responses received, 52% of the contact cases (n=109) were declared as laboratory-confirmed at the time the immunoglobulins were delivered. When there had been a laboratory confirmation, the type of confirmation was requested in the questionnaire. The declared types of confirmation are described in Table 1. In eight cases declared as laboratory-confirmed, the type of confirmation written in the questionnaire was the rash, meaning that for these cases, the diagnosis was made using clinical signs, without laboratory confirmation. In three cases, the declared type of laboratory confirmation was rash+ELISA, meaning that the rash was used as clinical confirmation and the ELISA as the laboratory confirmation. In one case, the declared type of laboratory confirmation was salivary sample, which was an imprecise answer explaining what kind of sampling was done but not the laboratory test performed (both PCR and ELISA could have been done using the salivary sample). Consequently, removing the eight cases clinically confirmed based solely on the rash, only 101 cases (48%) were laboratory-confirmed at the time the immunoglobulins were delivered. These 101 cases include those for whom the type of confirmation was not specified, assuming they were likely to have been confirmed by laboratory methods. In 48 additional cases, the laboratory confirmation of the contact case arrived a few days after the administration of the immunoglobulins (this information had been added as a comment in the answers).

In Table 2, results are presented by patient category. The eight immunocompromised children under the age of six months were counted in both categories "children aged under six months" and "immunocompromised persons". The seven responses with missing data for the patient category do not appear in this Table. The eight cases declared as laboratory-confirmed but only clinically confirmed based on the rash were considered as unconfirmed in the following analysis (six were children under six months-old and two were children between 6 and 11 months-old).

**Table 1**

Declared type of laboratory confirmation for contacts of people receiving anti-measles immunoglobulins, metropolitan France, 1 January 2010 to 31 August 2011 (n=109)

| | PCR | ELISA | PCR+ELISA | Rash | Rash+ELISA | Salivary sample | NA |
|---|---|---|---|---|---|---|---|
| Number | 40 | 40 | 2 | 8 | 3 | 1 | 15 |
| Percentage | 37 | 37 | 1.8 | 7.3 | 2.8 | 0.9 | 14 |

ELISA: enzyme-linked immunosorbent assay; NA: not available; PCR: polymerase chain reaction.

**Table 2**

Sex ratio, post-exposure delay (mean and percentage of post-exposure delay less or equal than six days) and contact case confirmation by patient category, metropolitan France, 1 January 2010 to 31 August 2011 (n=393)

| | Children aged under six months (n=172) | Children aged between 6 and 11 months (n=55) | Pregnant women (n=47) | Immunocompromised persons | Others (n=7) |
|---|---|---|---|---|---|
| Sex ratio[a] (male/female) | 0.59 (102/70) | 0.49 (27/28) | - | 0.58 (69/51) | 0.29 (2/5) |
| Mean post-exposure delay in days (min/max) | 4.1 ± 2.2 (0/10) | 5.6 ± 4.3 (1/30) | 5.5 ± 0.3 (1/8) | 4.1 ± 3.7 (0/21) | 6.6 ± 0.4 (0/7) |
| Post-exposure delay ≤ 6 days % (n) | | | | | |
| Yes | 70.9 (122) | 58.2 (32) | 46.8 (22) | 32.5 (39) | 14.3 (1) |
| No | 8.1 (14) | 23.6 (13) | 4.3 (2) | 6.7 (8) | 57.1 (4) |
| Not available | 20.9 (36) | 18.2 (10) | 48.9 (23) | 60.8 (73) | 28.6 (2) |
| Laboratory confirmation of the contact case % (n) | | | | | |
| Yes | 20.9 (36) | 40.0 (22) | 29.8 (14) | 25.0 (30) | 57.1 (4) |
| No | 44.2 (76) | 20.0 (11) | 6.4 (3) | 16.7 (20) | 0 (0) |
| Not available | 34.9 (60) | 40.0 (22) | 63.8 (30) | 58.3 (70) | 42.9 (3) |

[a] The sex ratio corresponds to the number of men (or boys) divided by the total number of patients.

There were more men (or boys) among the children aged under six months and among the immunocompromised persons and more women in the category "others". The mean post-exposure delay in the different groups ranged from 4.1 days to 6.6 days. The delay was the smallest among children under the age of six months and among immunocompromised persons. In three cases, immunoglobulins were delivered very late (14 and 21 days after exposure for two immunocompromised persons and 30 days after exposure for one child between 6 and 11 months-old). To evaluate adherence to recommendations, we analysed this variable using two categories: post-exposure delay of six days or less (as recommended) and post-exposure delay more than six days (Table 2). Many responses were lacking data for this new variable, especially for immunocompromised persons (61%) and pregnant women (49%). The category for which most administrations occurred within six days after exposure was "children aged under six months". The category in which most administrations occurred more than six days after exposure was "others" (four of seven cases), but there were very few cases in this category. It was followed by "children aged between 6 and 11 months", with 13 of 55 cases. However, for eight of these 13 cases, immunoglobulins were delivered seven days after exposure (data not shown).

Regarding laboratory confirmation of the contact case, missing data ranged from 35% for children aged under six months to 64% for pregnant women. The percentage of confirmed cases was highest for the category "others", but because of the low number of cases in this category, we cannot consider this percentage as representative. For the category "children aged under six months", 76 of 172 cases (44%) were unconfirmed. Among the 172 children aged under six months, 27 (16%) were less than one week-old, and 10 were newborns. For the 112 cases for whom the origin of the exposure was known, exposure came from family members (including the mother) in 60% of the cases (n=66). Other cases were exposed through medical staff (n=45, i.e. 40%) or a nanny (n=1, i.e. 0.9%). Among pregnant women, the two most affected age groups were the 26–30 year-olds and the 31–35 year-olds (data not shown).

Among all 400 patients, we identified 10 children who possibly should have been vaccinated (children aged between 6 and 11 months for whom the post-exposure delay was three days or less) and one pregnant women who should not have received immunoglobulins (she had received two vaccination doses). The recommendations were not followed for these patients, but their medical conditions may have influenced the decision to deliver immunoglobulins.

## Discussion

This survey permitted an assessment of immunoglobulin delivery as post-exposure prophylaxis in the event of contact with measles between 1 January 2010 and 31 August 2011. We counted 400 administrations during the study period, with most cases being children under the age of one year.

The response rate of 73% was satisfactory for a postal survey. We could not contact every hospital pharmacy in metropolitan France because the French National

Council of the Order of Pharmacists did not provide an exhaustive list. According to the French hospital federation, there are 571 hospitals in metropolitan France (including major and minor hospitals, not local ones) [15]. Therefore, we contacted 65% of French major and minor hospitals but no local hospitals. However, we sent the questionnaire to 80% of France's major hospitals, which are more likely to deliver immunoglobulins in the studied indications. Therefore, we consider our study to be representative.

One of the objectives of this survey was to describe characteristics of patients having received prophylactic immunoglobulins following exposure to a measles case. The recommendations related to preventive measures to be implemented in the event of a measles case or cluster had been issued in 2005 and updated in 2009 and thus predated the current epidemic in France. Since then, no evaluation of the application of this measure has been conducted. With the current epidemic, basic information on post-exposure delay and percentage of confirmed contact cases could be important for decision makers in planning awareness campaigns or adapting recommendations. We therefore opted for a simple questionnaire, excluding questions about clinical diagnosis, the quantity of immunoglobulins delivered or patient evolution, so as to achieve a good response rate.

Consequently, we describe here the basic characteristics of the patients affected by these prophylactic measures. These data complement those obtained by notification, describing cases exposed to measles. The characteristics of the patients were different from those with a measles infection. Firstly, children under the age of one year, who are the age group most affected by measles infection, were proportionally even more represented among those receiving prophylaxis. Secondly, the most represented age group for adults receiving prophylaxis were the 26 to 30 year-olds, while the adult group most affected by measles infection are the 15 to 19 year-olds. Lastly, the age range of people affected by the prophylactic measures was wider than the age range of measles cases. This is linked to the categories of people for whom immunoglobulin delivery was recommended in this indication.

As previously stated, children under the age of one year were the most affected, especially very young children. Measles in infants can be very dangerous, and complications are very frequent in this age group (diarrhoea, otitis media, pneumonia, increased risk of encephalitis and panencephalitis, etc.) [1]. There are still cases of measles among women giving birth, which puts newborns at risk for subacute sclerosing panencephalitis, a fatal disease that manifests months or years after exposure [16]. Most children aged under six months for whom the source of exposure was known, were exposed by family members or medical staff. It is therefore crucial to increase awareness of measles among parents and medical staff [17]. Information campaigns directed towards women of child-bearing age or future parents could be useful in reducing the number of cases among pregnant women and newborns. Awareness campaigns could also be organised for medical staff working with immunocompromised persons.

Our results regarding temporal and geographic pattern are consistent with data from the French Institute for Public Health Surveillance (InVS) on the measles epidemic in France [9] which also show a concentration of the cases in south-eastern France. To address the concentration in this area, specific local actions might be useful. The incidence peak of immunoglobulin administrations occurred in March and April 2011, as did the incidence peak of measles cases. Since 2008, the epidemic has increased each year until 2011 and slowed down in 2012. Despite the decrease in the number of cases in 2012, authorities should continue promoting measles vaccination to control the spread of measles and end this epidemic.

The second objective of this survey was to describe practices related to post-exposure delay and laboratory confirmation of contact cases. The recommendation regarding the timing of immunoglobulins delivery was well followed: most administrations occurred within six days after exposure. However, a large proportion of the responses had missing or imprecise data for this question, highlighting the difficulty to estimate this post-exposure delay. In at least one case, the pharmacist (and the doctor) took the appearance of the rash as the beginning of exposure. Yet the contagious period usually begins five days before appearance of the rash, making it more difficult to estimate the beginning of exposure. In three cases, the immunoglobulins were delivered very late (14, 21 and 30 days after exposure). The efficiency of immunoglobulins decreases strongly after six days following exposure. At this point, exposed persons would already be sick if they were infected by measles. Additional explanations regarding the inefficiency of immunoglobulins after six days of exposure might have to be added to the current recommendations.

Only half of the contact cases were laboratory-confirmed when the immunoglobulins were delivered. However, in 48 cases, the results of laboratory analyses were known a few days after immunoglobulin delivery, demonstrating the willingness of hospital teams to confirm contact cases. In these situations, the immunoglobulins must have been delivered for medical reasons justifying the absence of results. As this prophylactic measure concerns fragile persons who cannot be vaccinated after exposure, the precautionary principle may have prevailed in order to avoid potentially severe complications. For the category "children aged under six months", 44% of the contact cases were not laboratory-confirmed at the time the immunoglobulins were delivered. The short mean post-exposure delay of 4.1 days is consistent with the hypothesis of rapid delivery to avoid complications. A more precise

survey with access to medical files could identify the reasons for immunoglobulin delivery in the absence of laboratory results. One of the reasons could be delays in running the laboratory analyses. Furthermore, during an epidemic like the current one in France, the positive predictive value of clinical diagnosis is quite good, so that not every contact case requires confirmation. Recommendations were made before the current outbreak, when the number of measles cases was low. In light of the current measles epidemic, these recommendations may need to be updated.

The results obtained in our survey on the characteristics of persons affected by anti-measles prophylaxis and of the pattern of exposure will help implementing actions targeted at specific categories of the population to control measles epidemic in France. The description of the post-exposure delay and of the percentage of contact cases confirmed may be useful for decision makers in adapting the recommendations to the current situation.

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