Meeting for the evaluation of alternative sampling methods for measles and rubella case confirmation held on 14th July 2004.

Conclusions and recommendations

- The meeting reviewed the results of recent studies in Uganda, Côte d'Ivoire and Turkey designed to evaluate the performance of dried blood spots and oral fluid samples for measles surveillance. The results of these studies were encouraging and confirm results from earlier research studies. Taken together, the results support the case for wider use of alternative methods for measles / rubella surveillance.
- Full details of the preliminary results are given in the meeting papers. Key findings were
    1. Dried blood spots (DBS) were straightforward to collect and process.
    2. High sensitivity (97-100%) and specificity (91.4-100%) of measles IgM detection in DBS compared to serum. In the Turkish studies, rubella IgM detection in DBS was accurate in a small panel of 65 samples..
    3. Measles RNA was not detected on filter papers extracts when standard RT-PCR assays were used. Results from nested RT-PCR assays were variable in the Ugandan studies, but more consistent in the Côte d'Ivoire study. Some technical issues remain to be resolved about the amount of DBS to extract and optimum RT-PCR assay to use.
    4. Oral fluid collection, processing and testing was straightforward in all studies.
    5. Promising results for measles IgM detection in oral fluid samples (OFS) were obtained; sensitivity (96.1-99.2%), specificity (83.5-100%).
    6. A high rate of detection of measles virus RNA in OFS by RT-PCR was achieved in the Ugandan study when a standard (ie. single step) RT-PCR assay was used. PCR testing of OF samples from the Turkey and Cõte d'Ivoire studies has yet to be completed.
    7. A range of key issues for implementing alternative samples for surveillance were discussed including training, availability of tests and costs. Although there is limited data on the performance of rubella IgM tests, the wider use of DBS was recommended for surveillance purposes.. OFS have performed well in these studies, but the increased costs of collection devices and assays, and the lack of a commercial rubella IgM test for OFS mean that this approach cannot be widely adopted at present.

Recommendations

1. The following recommendations for implementation were agreed for the different stages of measles control. Although IgM detection in serum samples is still considered the "gold standard" for measles and rubella case confirmation:
    - Regions that <u>have not controlled measles</u>, and
        - Continue to experience measles (rash illness and fever) outbreaks in remote regions, and
        - Have minimal laboratory support, and
        - Have only have limited access to required expertise / supplies for conventional specimen collection.

      May benefit from the ease of DBS or OF sampling methods (if reverse cold chain is available). Use of alternative sampling methods without

collection of companion conventional sampling methods (blood and throat swabs, etc.) is to be encouraged, particularly in countries or geographic regions where little or no information concerning rash illness and fever outbreaks has been available. However, when possible, conventional sampling methods should be performed in a predetermined percentage of suspect cases.

- Regions that have controlled measles, but
  - Continue to have periodic outbreaks of measles, and
  - Have a well developed sub-national laboratory network, and
  - Could initiate DBS or OF sampling in parallel with conventional methods for a predetermined percentage of suspect cases.
- Regions that have controlled measles and are in an elimination phase of the program, and
  - Have moved to case-based surveillance, and
  - Have very infrequent outbreaks of measles via importation from endemic countries

  Probably would not benefit from DBS or OF sampling methods, but perhaps should be discussed on a case-by-case basis (e.g., where there are areas in a country which report clinical cases but do not have the ability to collect conventional samples).
  - Note: Obvious exception is the U.K., where OF is used primarily as a passive surveillance tool and where they have the ability to obtain a second confirmatory sample from IgM-positive persons.

- The meeting identified 3 functional applications where alternative samples might play a role in measles surveillance
  - The results using DBS are compatible with serum but operational studies are required to define issues for effective implementation including some country-based parallel studies. WHO regions are asked to identify sites to study these issues over the next 12 months with a view to reviewing operational performance before widespread routine application.
  - DBS have a valuable complimentary role to serum based surveillance in difficult to reach populations (lack of cold chain etc)
  - In some settings, supplementary collection of oral fluid samples for PCR and genetic characterisation may enhance overall surveillance.
- **Action: WHO HQ and WHO ROs.**

2. The collection of OFS poses minimal risk to patient or health staff collecting the sample. However the meeting identified areas of risk in collecting DBS unless sterile, single use lancets and gloves are provided to staff involved with sample collection.
   - Sample collection kits comprising single use sterile lancets, gloves, filter paper, sealable envelope, desiccator packet, and clear instructions should be provided to all health facilities involved in DBS sampling procedures.
   - Training of health staff in DBS collection methods will be required to reduce the risk of blood borne disease transmission for patient and health worker.
   - **Action: WHO HQ and WHO ROs.**

3. The meeting recognised that the Turkish evaluation of rubella IgM detection in DBS showed strong correlation with results from companion serum samples. However further validation with larger sample size is appropriate.
    - Comparison of rubella IgM in DBS and OFS with serum samples should be further evaluated. The usefulness of using molecular techniques for detecting rubella virus in these samples should also be encouraged.
    - **Action: WHO HQ and Global Specialised Labs**

4. Evidence was presented on the stability of IgM and measles RNA in DBS and OFS, and ambient temperature transportation is an attractive feature of these sampling procedures. However the meeting recognised that limited data were available for the high ambient temperatures experienced in many of the countries where alternative sampling techniques may be considered more practical than serum.
    - The stability and longevity of measles and rubella IgM and RNA should be investigated, especially at temperatures in the range of 30-40°C.
    - **Action: WHO HQ and Global Specialised Labs**

5. The current protocols for collection and testing of DBS and OFS appear to be robust and easy to follow. However, minor modifications to the current protocol for collection and extraction of DBS will be required to provide sufficient sample volume for measles IgM testing, to perform rubella testing if the measles IgM test is negative, and to allow validation of results by the appropriate reference laboratory. The protocol should also include a list of the strengths and limitations of each technique so that decisions of use are based upon complete information (e.g., the limitation in the ability to test for differential diagnoses (DBS & OFS), limited volumes of sample (DBS) available for multiple tests etc.)
    - **Action: WHO HQ and Global Specialised Labs**