DETECTION OF MEASLES AND RUBELLA ANTIBODIES IN DRIED BLOOD SPOTS

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ABSTRACT
This study aimed to determine the frequency of detection of measles and rubella antibodies in dried blood spots in Bulgaria.

Material and Methods: Two types of clinical material, serum samples and dried blood spots (DBS), were tested from a total of 101 patients. Serological methods (indirect ELISA) were used for detection of specific viral markers (IgM and IgG antibodies) indicating acute or past measles and rubella infection.

Results: In the present study, the patients were with median age of 39 years and divided into 11 age groups. The majority of patients were under 30 years of age and from the capital of Sofia. In 3 patients acute measles infection was confirmed by positive ELISA-IgM results for the serum samples and DBS. No acute rubella infection was detected. Measles and rubella IgG seroprevalence was determined as 83/101 (82%, 95% CI: 74.51÷89.49) and 79/101 (78%, 95% CI: 69.92÷86.08) in serum samples, and 79/101 (78%, 95% CI: 69.92÷86.08) and 73/101 (72%, 95% CI: 63.25÷80.75) in DBS, respectively.

In combination with immunoenzymatic testing for measles and rubella IgM/IgG markers, coincidence of results for both types of clinical material was found in >90% of cases. No significant differences were found in the results in terms of gender and age.

Conclusion: In recent years a variety of new and innovative applications of DBS are introduced in medicine, neonatology, virology, microbiology, etc. The optimisation of the DBS technique as an alternative approach to venepuncture in virology is very important for conducting seroepidemiological studies and to a certain extent for the surveillance of epidemic outbreaks.

KEYWORDS: dried blood spots, measles, rubella, ELISA assay, IgM/IgG antibodies

INTRODUCTION
Ivar Christian Bang (1869-1918) is considered the founder of modern clinical microanalysis (1, 2) and the idea of using blood collected on paper map made of cellulose and filter paper technique. Subsequently, several researchers reported the use of dry blood spots (DBS) in serological tests for the diagnosis of syphilis (2), for the detection of antibodies against measles, mumps, polio virus, parainfluenza virus and respiratory syncytial virus in 1953 (2), and also for the identification of Shigella in stool (faeces) dried on filter paper (3). In 1924, Chapman (4) summarises the advantages of DBS technique, stressing four key points that are still valid today: (a) compared to conventional venepuncture, it requires less blood volume, which is especially important in areas such as pediatrics and neonatology; (b) blood collection procedure is easy, inexpensive and non-invasive; (c) the risk of bacterial contamination or haemolysis is minimal; and (d) DBS can be maintained for a long time with almost no impact on the quality of the analysis. In 1953 the application of this technique is considered for detection of syphilis, antibodies against measles, mumps, polio virus, parainfluenza virus and respiratory syncytial virus (2) and also for the identification of Shigella in stool dried on filter paper (3). In 1969/70s Guthrie published his method of neonatal screening for phenylketonuria with DBS obtained by prickling the heel of newborns (5) and surveillance of congenital
hypothyroidism and sickle cell disorders (5, 6). The approach of using capillary blood obtained from heel or finger and soaked on filter paper, was conducted to screen for metabolic disease in a large population of newborns in Scotland in 1963 (5).

There are many reports on the application of DBS in medicine, toxicology, pharmacokinetics, metabolic exchange, therapeutic drug monitoring, forensic toxicology studies, in clinical laboratory diagnostics and chemistry (7, 8).

Viral infections weaken the immune system and open the door to secondary health problems such as pneumonia, blindness, diarrhoea, encephalitis, etc. The main methods for diagnosis of viral infections are based on detection of specific antiviral antibodies in blood specimens. Measles and rubella infections are vaccine-preventable diseases and are a major cause of morbidity and mortality in children worldwide. The high contagious index (>90% for measles and rubella, and >50% for mumps) and the occurrence of debilitating complications with high frequency determine their health and socioeconomic importance. Approximately 30% of reported measles cases have one or more complications, such as disabling effects that are most common in children under 5 years of age. The importance of rubella infection for the public health is determined by the teratogenic effects of the virus during pregnancy. There is high percentage of miscarriages, stillbirths or congenital rubella syndrome.

The collection of blood, particularly from children, is very often difficult and transportation of the samples to the laboratory in a cold chain is not always achievable. In these cases using DBS as an alternative sampling technique is suitable. DBS have been used for a range of epidemiological studies as an alternative to serum. Antibodies are stable in this form and therefore this method is particularly valuable where the lack of a cold chain is an issue. DBS technique has recently been applied successfully in measles cases, and there is accumulating evidence that it will work for rubella cases as well (9, 10, 11).

This study aimed to determine the frequency of evidence of measles and rubella antibodies in dried blood spots in Bulgaria.

**MATERIAL AND METHODS**

**Material - Study Area and Sample Collection**

Two types of clinical material, serum samples and dried blood spots, were tested from a total of 101 patients. The specimens were collected under the terms of a research project funded by the National Science Fund, Bulgaria, Contract № DM 03/1,12.12.2016, and tested at the National Reference Laboratory (NRL) “Measles, Mumps and Rubella” of the National Centre of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria. Blood specimens were collected from all patients by venepuncture and by pricking a finger or heel using sterile automatic lancets for preparation of dried blood spots (1.5 - 2 mm). Blood was centrifuged at 4000 x g for 10 minutes and the serum was aliquoted and frozen at -20°C until analysed. Blood spots were stored on the cards of filter paper, labelled, dried at room temperature for 30 minutes and put in ziploc bags with desiccant for storage at 2°C - 8°C.

The median age was 39 years. The majority of patients were under 30 years of age and from the capital of Sofia.

**Methods**

· **Serological analysis**

All specimens were tested for presence of anti-measles IgM/IgG and anti-rubella IgM/IgG antibodies with a commercial indirect enzyme-linked immunosorbent assay (Anti-Measles IgM/IgG ELISA, Euroimmun, Germany and Anti-Rubella, IgG/IgM, Euroimmun, Germany). The tests were carried out according to the manufacturer’s instructions. The absorbance values were divided by the mean absorbance values of cutoff calibrator and the results were interpreted qualitatively as positive, negative or equivocal.

· **Statistical Analysis**

For the statistical processing of the results obtained we used relative percentages (%), confidence interval (95% CI), graphical and table analysis.

**RESULTS**

In the present study, the tested patients were aged from 1 to 82 years with median age of 39 years. Due to this wide range they were divided
in 11 age groups (Figure 1). The majority of patients were under 30 years of age and from Sofia (62/101, 61.38%, 95% CI: 51.88÷70.88) and Burgas regions (18/101, 17.82%, 95% CI: 10.36÷25.28). The rest were from the regions of Plovdiv, Stara Zagora, Pazardzhik and Montana.

In 3 patients acute measles infection was confirmed with ELISA-IgM test of the serum samples and DBS. No acute rubella infection and IgM viral marker were detected. The obtained results corresponded with the clinical manifestation, as 12 out of 101 patients were diagnosed with a possible measles infection and none with rubella infection. The samples were collected using case-based surveillance of these infections in Bulgaria. Immunoassay analysis of all 101 patients showed the presence of measles and rubella IgG in 83/101 (82%, 95% CI: 74.51÷89.49) and 79/101 (78%, 95% CI: 69.92÷86.08) serum samples, and in 79/101 (78%, 95% CI: 69.92÷86.08) and 73/101 (72%, 95% CI: 63.25÷80.75) DBS, respectively (Figure 2).

Figure 1. Age group distribution of the patients (n=101).

Figure 2. Frequency of detection of immunoenzymatic measles and rubella diagnostic markers (ELISA IgM/IgG) in serum and DBS.
The calculated percentage for coincidence of results obtained with the two types of clinical materials was more than 90%. Different ELISA IgG results were found for 4 DBS tested as negative for measles and rubella, compared with positive results of the serum samples from the same person. When determining the frequency of viral markers detection in DBS and serum samples, no significant differences in the results were found in terms of gender and age of patients. DBS collection was successful in young children and infants as well as in adults, including pregnant women, which is useful for their application in clinical practice.

DISCUSSION
In the beginning of the 21st century, the world, Europe and Bulgaria are taking the path of elimination of measles and rubella (12). This process is difficult to implement because of many epidemic outbreaks and low immunisation coverage of certain population groups. According to European Centre for Disease Prevention and Control (ECDC) data, between 1 July 2017 and 30 June 2018, 29 EU/EEA Member States reported 13 234 cases of measles. During the period, most cases were reported by Italy (3 341), Greece (3 193), France (2 740) and Romania (1 354), accounting for 25%, 24%, 21% and 10%, respectively, of all cases reported by EU/EEA countries (13). Between 1 July 2017 and 30 June 2018, 13 EU/EEA Member States reported a total of 624 cases of rubella with Poland (490), Germany (60), Italy (27) and Austria (21) (13). Collecting venous blood samples is the "gold standard" in laboratory detection of measles and rubella. The collection of blood samples, particularly from children, is a problem as it is an invasive technique, often creates discomfort (pain, injection vacuum system, trained staff, etc.) and maintaining a cold chain when transporting samples to the laboratory is not always achievable. This necessitates the search for alternative samples (dried blood spots and oral fluid) and techniques to detect the viruses and the specific antibodies against them (11).

Recently in the WHO Measles and Rubella Laboratory Network the use of DBS begins to be validated as a useful tool alternative to serum for the measles/rubella program in a range of epidemiological studies (10, 14, 15, 16). The viral antibody remains stable in DBS which is particularly valuable where the lack of a cold chain and logistics are an issue. Helfand et al. (16) and Karapanagiotidis et al. (10) reported a successful application of this technique in measles cases, and there is accumulating evidence that it will work for rubella cases as well. Uzicanin et al. (17) and Helfand et al. (9) published studies on the seroepidemiological importance of DBS in the control and monitoring of measles and rubella infection. The authors prove DBS as a real alternative to serum samples, particularly in epidemic situations. Our results confirm the potential use of this technique in the laboratory diagnosis of acute and past (presence of protective immunity) measles and rubella infection. In combination with immunoenzymatic testing of measles and rubella IgM/IgG markers, coincidence of results for both types of clinical materials was found in >90% of cases. The study covered a two-year period in which 2 measles outbreaks and 0 rubella cases were reported in Bulgaria. Patient samples were selectively collected, mainly from healthy vaccinated people. Despite the comparatively small number of tested samples, the study showed the potential role of DBS in proving viral markers with standard immunoassay, and optimised the serum elution protocol. The study focuses on the enrichment of the laboratory range for detection of measles and rubella virus in Bulgaria and provides an easy-to-carry test of the immune status of the population in the phase of elimination of these infections.

CONCLUSION
DBS approach is non-aggressive and more acceptable to the public, including young children, pregnant women, etc. In recent years a variety of new and innovative applications of DBS are introduced in medicine, neonatology, virology, microbiology, etc. The optimisation of DBS technique as an alternative approach (non-invasive, inexpensive, not requiring trained staff and cold chain for transport and storage) to venepuncture in virology, is very important for conducting of seroepidemiological studies and to a certain extent for the surveillance of epidemic
outbreaks. Serum-based technology remains a major approach in the immunoenzymatic diagnosis of viral infections.

Competing Interest
The authors do not have any competing interest.

ACKNOWLEDGEMENTS
This study was supported by project № DM 03/1, 12.12.16 “Dried blood spots (DBS) as alternative, archival material for detection of viral agents (measles, mumps, rubella, hepatitis B virus) in Bulgarian population”, „Financial Support Competition of Junior Researchers Projects – 2016”, National Science Fund, Bulgaria.

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