H. Vaccine-Preventable Diseases

Measles, Mumps, Rubella

Gutiérrez, Rodríguez MJ, DeOry F, Piédrola G, Maroto MC. Reliability of low-avidity IgG and of IgA in the diagnosis of primary infection by rubella virus with adaptation of a commercial test. Journal of Clinical Laboratory Analysis 1999;13:1-4

Perry KR, Brown DWG, Parry JV, Panday S, Pipkin C, Richards A. Detection of measles, mumps, and rubella antibodies in saliva using antibody capture radioimmunoassay. Journal of Medical Virology 1993;40:235-40

Ramsay ME, Brugha R, Brown DWG, Cohen BJ, Miller E. Salivary diagnosis of rubella: a study of notified cases in the United Kingdom, 1991-4. Epidemiology and Infection 1998;120:315-9

Takahashi S, Machikawa F, Noda A, Oda T, Tachikawa T. Detection of immunoglobulin G and A antibodies to rubella virus in urine and antibody responses to vaccine-induced infection. Clinical and Diagnostic Laboratory Immunology 1998;5(1):24-7

Thieme T, Piacentini S, Davidson S, Steingart K. Determination of measles, mumps, and rubella immunization status using oral fluid samples. Journal of the American Medical Association 1994;272(3):219-21

Author:	**Gutiérrez, Rodríguez MJ, DeOry F, Piédrola G, Maroto MC**
Title:	**Reliability of low-avidity IgG and of IgA in the diagnosis of primary infection by rubella virus with adaptation of a commercial test**
Source:	**Journal of Clinical Laboratory Analysis 1999;13:1-4**

This project studied the diagnostic reliability of IgG avidity and the presence of IgA for the diagnosis of recent infection by rubella virus. The examiners used a commercial test (Avy-EIA) using 8 molar urea (indirect ELISA) to determine low avidity IgG and a homemade indirect ELISA to determine IgA. Twenty-five patients with recent primary infection (group I) by rubella virus and 50 healthy subjects (group II) participated in the study. In group I, the percentages of low-avidity IgG varied between 100 and 0%; IgA was present in 24 (96%) patients. In group II, the percentages low avidity IgG varied from 50.4 to 0%; IgA was present in 2 (4%) subjects who had previously had an acute infection by rubella virus within the previous six months. The Avi-EIA had a sensitivity of the 92% and a specificity of 100%. For the IgA test, sensitivity was 96% and specificity 96%. Both Avi-EIA and IgA tests are simple and reliable for the diagnosis of recent primary infection. Nevertheless, the IgG avidity and IgA assays should be considered as complementary methods to the determination of IgG and IgM, and the clinical situation of the patient must be taken into account to avoid false negative and false positive results.

Author:	**Perry KR, Brown DWG, Parry JV, Panday S, Pipkin C, Richards A**
Title:	**Detection of measles, mumps, and rubella antibodies in saliva using antibody capture radioimmunoassay**
Source:	**Journal of Medical Virology 1993;40:235-40**

This study was designed to investigate the salivary diagnosis for measles, mumps, and rubella. Saliva specimens were collected from patients with clinically and serologically confirmed measles, mumps and rubella and tested by antibody capture assays for virus specific IgM and IgG. This paper reports on the sensitivity and specificity of these assays for detecting salivary IgM and IgG responses and makes recommendations for optimum sampling times following onset of symptoms. Simultaneously collected serum and saliva specimens from healthy blood donors were tested for measles (88), mumps (88), and rubella (91) specific IgM. Saliva was collected from serologically confirmed cases of measles (63), mumps (19), and rubella (150) and tested for virus specific IgG and IgM. Cases were confirmed serologically either by the detection of virus specific IgM in serum or by the demonstration of rising antibody titres in paired serum samples using complement fixation tests. In the first seven days following onset, infection was verified by salivary test in 92% of measles, 75% of mumps, and 100% of rubella cases. A few saliva specimens did not contain detectable virus specific IgM during the first week of illness, but it was detected in 100% of saliva specimens collected between one and five weeks after onset. After the fifth week, the proportion of reactive specimens declined. Specificity was established by testing saliva samples collected from blood donors. None of the saliva samples tested for measles or rubella reacted, but 1 of 88 specimens for mumps specific IgM contained significant activity. Virus specific IgM can be reliably detected in saliva samples collected from acute cases of measles, mumps, and rubella and identified 1-5 weeks after onset of illness.

Author:	**Ramsay ME, Brugha R, Brown DWG, Cohen BJ, Miller E**
Title:	**Salivary diagnosis of rubella: a study of notified cases in the United Kingdom, 1991-4**
Source:	**Epidemiology and Infection 1998;120:315-9**

This study was designed to assess the sensitivity and specificity of salivary rubella-specific IgM and IgG in comparison to blood testing for the detection of rubella. The researchers collected blood samples from 180 notified rubella cases, 54 of which were rubella IgM positive. Two of these cases had been vaccinated after the onset of disease and were excluded from the study. Therefore, 52 (29%) of the 178 cases were rubella IgM positive by blood testing. Paired blood and saliva were available for 177 of the 178 cases; 166 (94%) of the 177 paired samples gave the same results. The saliva IgM test had a sensitivity of 81% and specificity of 99%. The test had positive predictive value of 98% and negative predictive vale of 92%. The saliva IgG test had a sensitivity of 98% and specificity of 100%. Of 126 rubella IgM negative cases, 25 (20%) were positive for parvovirus B19 IgM. A surveillance system based on clinical reporting alone may provide biased epidemiological data, especially in younger children. Finally, saliva samples should be taken 7-42 days after onset of illness and transported rapidly to the laboratory for testing.

Author:	**Takahashi S, Machikawa F, Noda A, Oda T, Tachikawa T**
Title:	**Detection of immunoglobulin G and A antibodies to rubella virus in urine and antibody responses to vaccine-induced infection**
Source:	**Clinical and Diagnostic Laboratory Immunology 1998;5(1):24-7**

This research group developed an ELISA method for detection of anti-rubella virus (RV) IgG and IgA antibodies in human urine and compared its usefulness to that of a commercially available kit for serum antibody detection. 89 healthy volunteers (6 female and 83 male) provided urine and serum samples. In addition, three healthy individuals underwent rubella vaccination. The examiners used the new ELISA test to detect IgG, IgA, and IgM to rubella virus. 68 samples tested positive and 21 tested negative based on the results of the serum IgG assay. The urinary IgG assay had perfect (100%) sensitivity and specificity compared to serum IgG. However only 56 of 68 (82%) in the serum IgG assay positive group had urinary IgA positive results. Therefore, urinary IgG, but not IgA, was useful for screening. The three adults who underwent rubella vaccination had their levels of urinary IgG and IgA antibody and serum IgG and IgM antibody against RV measured. The serum and urinary IgG levels remained elevated from the third or fourth week after vaccination; serum IgM and urinary IgA levels rose rapidly between weeks three and five and then decreased slowly. The authors conclude that this simple ELISA detects urinary IgG and IgA antibodies to RV. Findings suggest that direct testing of urine samples for RV-specific IgG provides an efficient alternative to serum-based assays. These urine assays should be useful for screening for RV-specific antibody and diagnosing recent RV infection.

Author:	**Thieme T, Piacentini S, Davidson S, Steingart K**
Title:	**Determination of measles, mumps, and rubella immunization status using oral fluid samples**
Source:	**Journal of the American Medical Association 1994;272(3):219-21**

This study investigated the practicality of using an oral fluid collection device to obtain samples for monitoring the serostatus of people vaccinated against measles, mumps, and rubella viruses. 157 asymptomatic volunteers (55 women and 102 men) participated in the study by giving oral fluid samples in a patented device called OraSure and serum sample. In addition, a longitudinal study was undertaken in which 11 subjects (4 women and 7 men) with inadequate measles antibody levels elected to receive the measles-mumps-rubella vaccine and then volunteered to give serial matching blood and oral fluid samples for 180 days.

	All subjects		Subjects under 18	
	sensitivity	specificity	sensitivity	specificity
measles	96.7%	100%	100%	100%
mumps	94.2%	93.9%	75.0%	97.0%
rubella	98.0%	98.0%	87.0%	100%

In the longitudinal study, 8 of the 11 subjects seroconverted to protective measles antibody levels within 180 days after vaccination. The mean time of measles seroconversion measured by serum was 18.3 days and by oral fluid 18.8 days. In conclusion, the simple and noninvasive nature of the collection procedure makes testing for antiviral antibodies in oral fluid practical for use with pediatric subjects and in settings where blood sampling is inconvenient or impossible.

Pertussis

Buck GE. Detection of *Bordetella pertussis* by rapid-cycle PCR and colorimetric microwell hybridization. Journal of Clinical Microbiology 1996;34(6):1355-8

Cimolai N, Trombley C, O'Neill D. Diagnosis of whooping cough: a new era with rapid molecular diagnostics. Pediatric Emergency Care 1996;12(2):91-3

Erlandsson A, Bäckman A, Törnqvist E, Olsen P. PCR assay or culture for diagnosis of *Bordetella pertussis* in the routine diagnostic laboratory? Journal of Infection 1997;35:221-4

Grimprel E, Njamkepo E, Bégué P, Guiso N. Rapid diagnosis of pertussis in young infants: comparison of culture, PCR, and infant's and mother's serology. Clinical and Diagnostic Laboratory Immunology 1997;4(6):723-6

Müller F-M C, Schnitzler N, Cloot O, Kockelkorn P, Haase G, Li Z. The rationale and method for constructing internal control DNA used in pertussis polymerase chain reaction. Diagnostic Microbiology and Infectious Disease 1998;31:517-23

Müller F-M C, Heininger U, Schnitzler N, Kockelkorn P, Cloot O, Lorenz C, Haase G. Discrimination of *Bordetella parapertussis* and *Bodetella pertussis* organisms from clinical isolates by PCR using biotin-labelled oligonucleotide probes. Molecular and Cellular Probes 1998;12:2313-7

Author:	**Buck GE**
Title:	**Detection of *Bordetella pertussis* by rapid-cycle PCR and colorimetric microwell hybridization**
Source:	**Journal of Clinical Microbiology 1996;34(6):1355-8**

This study sought to design a highly sensitive and practical method for detecting and amplifying *Bordetella pertussis*. It combines a rapid temperature cycle system that uses recirculating hot air with a colorimetric microwell detection procedure for the rapid and convenient detection of *B. pertussis* by PCR. The combination of a rapid temperature transition rate, small sample volume, and overshooting or undershooting of the temperature set points allows the cycles to be reduced to 5 seconds for denaturation and 10 seconds for extension and anneating; thus the amplification can be completed in approximately 35 minutes. The results presented suggest that PCR is a rapid and highly sensitive means for detecting *B. pertussis* in clinical specimens. By this procedure, the total process, including specimen preparation, amplification, and detection, can be accomplished in about 2.5 hours. The combination of this rapid, convenient detection procedure with rapid-cycle PCR has several advantages. First, results are available quickly. Second, this procedure allows for more efficient use of reagents and equipment.

Author:	**Cimolai N, Trombley C, O'Neill D**
Title:	**Diagnosis of whooping cough: a new era with rapid molecular diagnostics**
Source:	**Pediatric Emergency Care 1996;12(2):91-3**

This paper describes a new rapid technique for the diagnosis of whooping cough using PCR. Examiners obtained nasopharyngeal washings from 456 children admitted to the emergency department or acute general pediatric clinic. Of the 456 samples assayed, the frequency of culture and initial PCR positivity was 9.2% and 14.5% respectively. Both culture and PCR were positive for 41 specimens (9%); 25 (5.5%) were positive by PCR but negative by culture. Contamination of negative controls did not occur. The data indicate that the frequency of positive lab diagnosis may be increased by as much as 30 to 40%. Identification of these additional infections may have considerable impact upon the suppression of active infection in the community and upon the identification of contacts who may receive chemoprophylaxis. The confirmation of PCR-positive, culture-negative specimens as true positives remains a concern, but there are several possible approaches that might address this issue.

Author:	**Erlandsson A, Bäckman A, Törnqvist E, Olsen P**
Title:	**PCR assay or culture for diagnosis of *Bordetella pertussis* in the routine diagnostic laboratory?**
Source:	**Journal of Infection 1997;35:221-4**

The aim of this study was to compare the nested PCR and culture for the diagnosis *of Bordetella pertussis*. 241 nasopharyngeal aspirates from consecutive patients with symptoms of pertussis served as specimens for the analysis. 83 samples tested positive by either the nested PCR or culture, and 158 tested negative by both.

		Culture		
		+	-	Total
Nested PCR	+	74	8	82

	-	1	158	159
	Total	75	166	241

The mean time to obtain diagnosis from arrival of the sample was 1.8 +-1.3 days for PCR, for positive cultures 4.5 +-1.4 days, and for negative cultures 10.5 +-1.0 days. The PCR required two hours of hands-on time, whereas a positive culture required 25 minutes and a negative culture 15 minutes. PCR had a sensitivity of 98.8%, and culture had a sensitivity of 90.4%. PCR has more material costs and requires more human labor time than cultures do. In addition, nested PCR requires separate rooms for handling of sample, reagents, and amplified material to avoid cross contamination of amplified materials. However, the authors still favor the use of PCR over culture due to its better sensitivity and rapidity.

Author: **Grimprel E, Njamkepo E, Bégué P, Guiso N**
Title: **Rapid diagnosis of pertussis in young infants: comparison of culture, PCR, and infant's and mother's serology**
Source: **Clinical and Diagnostic Laboratory Immunology 1997;4(6):723-6**

The aim of this study was to compare the contributions of culture, PCR, and compared pertussis serology in infants and their mothers, using prepartum serum, to the rapid diagnosis of pertussis. 28 infants less than 6 months with confirmed pertussis and their mothers who gave prepartum and acute sera participated in this study. Examiners collected a nasopharyngeal aspirate (NPA) for culture and PCR during the first visit for all infants and some mothers. 26 of the 28 infants (93%) had typical pertussis, prolonged cough lasting more than 21 days with peroxysms, and 2 had atypical pertussis, cough lasting less than 21 days. Of the mothers, 9 (32%) had typical pertussis, 9 (32%) had atypical pertussis, and 10 (36%) were asymptomatic. For the infants, the rates of positive culture was 43% (12 of 28) and positive PCR was 89% (25 of 28). For mothers, 22% (4 of 15) of cultures tested positive and 8 (53%) of PCRs. Culture and PCR sensitivity were 43% and 89%, so PCR sensitivity was twice that of culture for both infants and mothers. However, PCR was less sensitive for adults than for young infants, showing that PCR sensitivity decreases with the age of the patient. The authors propose a model for the diagnosis of pertussis in infants less than six months old. Although less sensitive than PCR, the serological method using prepartum serum should be used for a rapid diagnosis of pertussis in young infants when culture and PCR are either not available or negative.

Author: **Müller F-M C, Schnitzler N, Cloot O, Kockelkorn P, Haase G, Li Z**
Title: **The rationale and method for constructing internal control DNA used in pertussis polymerase chain reaction**
Source: **Diagnostic Microbiology and Infectious Disease 1998;31:517-23**

The inclusion of an appropriate internal control DNA in polymerase chain reaction (PCR) is a rapid and simple method for the detection of PCR failure. Their article describes simple approaches that should work under all circumstances to create internal control DNA with the same primer-building sequences as the target DNA by overlap extension technique. To interpret PCR results correctly it is critical to distinguish between PCR failure and real negative PCR results. This can easily be achieved by using coamplification controls. The ideal PCR internal contol should have the same primer-binding sequences as the target DNA. This study developed two different internal controls, the overlap extension technique and a method the generates ICD II

using PCR MIMICS. The examiners tested this ICD II in a prospective clinical study of 360 patients with clinical diagnosis of pertussis. The internal control was positive in 318 (88%) of the 360 nasopharyngeal swabs and was negative in 42 (12%). After phenolchloroform extraction, an additional ten internal controls became positive. The incorporation of an internal control DNA is highly recommended by has not yet become a standard in pertussis PCR.

Author:	**Müller F-M C, Heininger U, Schnitzler N, Kockelkorn P, Cloot O, Lorenz C, Haase G,**
Title:	**Discrimination of *Bordetella parapertussis* and *Bordetella pertussis* organisms from clinical isolates by PCR using biotin-labelled oligonucleotide probes**
Source:	**Molecular and Cellular Probes 1998;12:2313-7**

The aim of this study was to validate the recently described shared-primer PCR method which allows the simultaneous detection of *B. pertussis* and *B. parapertussis* organisms but cannot distinguish *B. parapertussis* from *B. bronchiseptica* organisms. Examiners collected 132 nasopharyngeal specimens from patients with a history of cough greater than seven days duration. After obtaining PCR results, culture results were unblinded for comparison. Of the 83 samples containing *B. parapertussis*, all 83 gave positive results for the *B. parapertussis*-specific probe. Of the 33 samples with *B. pertussis*, all 33 gave positive results with the *B. pertussis*-specific probe. All 16 negative controls with H. influenza gave results under the threshold for both probes. The advantage of PCR is most prominent in patients with previous immunizations against pertussis and in those who received one or more doses of antibiotics before the nasopharyngeal swab was taken. The higher sensitivity and more rapid results of PCR may outweigh its higher cost in comparison to culture. For the identification and discrimination *of B. pertussis* and *B. parapertussis* organisms, the shared primer PCR in combination with specific oligonucleotide probes is a rapid, sensitive, and specific molecular tool that may be used for future surveillance studies. It may be used to further investigate whether *B. parapertussis* antigens should be added to acellular pertussis vaccines to protect against *B. parapertussis* infections.

Poliomyelitis

Abraham R, Chonmaitree T, McCombs J, Prabhakar B, Lo Verde PT, Ogra PL. Rapid detection of poliovirus by reverse transcription and polymerase chain amplification: application for differentiation between poliovirus and nonpoliovirus enteroviruses. Journal of Clinical Microbiology 1993;31(2):395-9

Chezzi C. Rapid diagnosis of poliovirus infection by PCR amplification. Journal of Clinical Microbiology 1996;34(7):1722-5

Rifonan AS, Mann L, Chonmaitree T. Use of monoclonal antibodies to identify serotypes of enterovirus isolates. Journal of Clinical Microbiology 1998;36(7):1877-81

Schweiger B, Schreier E, Böthig B, López-Pila JM. Differentiation of vaccine and wild-type polioviruses using polymerase chain reaction and restriction enzyme analysis. Archives of Virology 1994;134:39-50

Author:	**Abraham R, Chonmaitree T, McCombs J, Prabhakar B, Lo Verde PT, Ogra PL**
Title:	**Rapid detection of poliovirus by reverse transcription and polymerase chain amplification: application for differentiation between poliovirus and nonpoliovirus enteroviruses**
Source:	**Journal of Clinical Microbiology 1993;31(2):395-9**

This study selected two specific oligonucleotide primers from the conserved 5' noncoding region of poliovirus to investigate the potential use of PCR for the rapid differentiation of polioviruses from nonpoliovirus enteroviruses. The examiners collected throat and rectal specimens with swabs, cerebrospinal fluid, and stool specimens. Of the 144 clinical viral isolates collected, 81 were identified as poliovirus and 50 as nonpoliovirus enteroviruses. All 81 (100%) viral isolates identified as polioviruses by tissue culture neutralization and infectivity were also positive by the PCR. Two nonpolio enterovirus isolates gave positive reactions. This test had a sensitivity of 100% and specificity of 96%. The results of the test show that with a single set of primers selected in the conversed 5' noncoding region, it is possible to identify polioviruses rapidly and with high degrees of specificity and sensitivity. The study shows that rapid exclusion of shedding of vaccine polioviruses in clinical specimens can be done by using reverse transcriptase PCR. The Southern blot confirms that the PCR procedure can be used to definitely identify the presence of poliovirus. In summary, the results indicate that reverse transcriptase PCR can potentially be used for specific detection of polioviruses and rapid differentiation of poliovirus and nonpolio enteroviruses.

Author:	**Chezzi C**
Title:	**Rapid diagnosis of poliovirus infection by PCR amplification**
Source:	**Journal of Clinical Microbiology 1996;34(7):1722-5**

This report describes the rapid detection of poliovirus in a simple reverse transcriptase (RT) – PCR assay in which a single primer pair is used. By this technique, it is possible to identify polioviruses, to differentiate them from other enteroviruses within 24 hours, and to determine the genotypes of wild-type polioviruses by direct sequencing of the PCR products. The examiners subjected 125 enterovirus isolates identified as poliovirus by conventional typing technique and 39 nonpolio enteroviruses to RT-PCR with primers PVPCR1 and 2A. All 125 isolates (100%) typed as poliovirus by tissue culture neutralization were also positive by PCR. The ability of the PCR assay to detect polioviruses directly in clinical specimens was measured by amplifying RNA extracted from stool specimens. RNA was extracted from 15 stool specimens from which poliovirus had been isolated; all 25 were positive for poliovirus by PCR. This PCR assay is highly sensitive, detecting as few as 4 RFU of infectious virus, and can be performed directly on clinical material. The authors conclude "RT-PCR with primers PVPCR2 and 2A can be used to rapidly identify polioviruses and to differentiate them from nonpolio enteroviruses."

Author:	**Rifonan AS, Mann L, Chonmaitree T**
Title:	**Use of monoclonal antibodies to identify serotypes of enterovirus isolates**
Source:	**Journal of Clinical Microbiology 1998;36(7):1877-81**

This study compared the results of the immuno flourescence assay (IFA) and the standard neutralization technique for the serotype identification of enteroviruses. The researchers sought to use IFA to rapidly differentiate between polioviruses and nonpolioviruses. Their study

analyzed 291 enterovirus isolates, of which 234 were consecutive isolates grown from specimens; of the 234 isolates, 196 were nonpolio enterovirus isolates and 38 were poliovirus isolates.

IFA identified the serotypes of 73.9% (173 of 234) of the consecutive isolates and 71.4% (208 of 291) of all enterovirus isolates by yielding a positive staining result. The level of agreement between the IFA and neutralization tests was 92%. In order to differentiate between polioviruses and nonpoliovirus enteroviruses, monoclonal antibodies for only three types of polioviruses are needed. The authors summarize "the IFA procedure with blends of monoclonal antibodies directed at polioviruses, coxsackievirus type B, and six common serotypes of echoviruses identified the serotypes of 74% of consecutive clinical enterovirus isolates."

Author:	**Schweiger B, Schreier E, Böthig B, López-Pila JM**
Title:	**Differentiation of vaccine and wild-type polioviruses using polymerase chain reaction and restriction enzyme analysis**
Source:	**Archives of Virology 1994;134:39-50**

This study describes the RFLP assay based on the variable parts of the 5'-noncoding region (5'-NCR) of the poliovirus genome. The results obtained with a set of various restriction enzymes distinguish vaccine-like and wild-virus virus. This study investigated more than 60 virus isolates including prototype strains. The results obtained with a set of various restriction enzymes confirmed the presence of highly conserved cleavage sites for vaccine-derived isolates. The analysis of the wild strains showed that at least four out of five selected enzymes generated patterns not characteristic of the vaccine strains. This result emphasizes that the RFLP analysis of the 5'-NCR is a specific and reliable method for assessing the origin of field isolates. The authors state "the RFLP assay developed for the 5'-NCR is a useful approach to differentiate vaccine-related and wild-type polioviruses and is a valuable supplement to virological diagnostic procedures."