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Detection and Quantitation of Rubella IgG Antibody: Evaluation and Performance Criteria for Multiple Component Test Products, Specimen Handling, and Use of Test Products in the Clinical Laboratory; Approved Guideline



This guideline identifies performance specifications and criteria for products used to detect rubella antibody. It also provides procedures for collecting and handling specimens submitted for rubella serological testing as well as the evaluation and reporting of results.



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Detection and Quantitation of Rubella IgG Antibody: Evaluation and Performance Criteria for Multiple Component Test Products, Specimen Handling, and Use of Test Products in the Clinical Laboratory; Approved Guideline

Abstract

Detection and Quantitation of Rubella IgG Antibody: Evaluation and Performance Criteria for Multiple Component Test Products, Specimen Handling, and Use of Test Products in the Clinical Laboratory; Approved Guideline (NCCLS document I/LA6-A) is presented in two parts in order to provide a complete review of rubella serology testing. Part I is intended for use primarily by manufacturers to enable them to meet the general product criteria outlined in Section 3, the general performance criteria and specific performance criteria (Sections 4 and 5, respectively), and to implement the validation testing procedures presented in Section 6. Part I should also be useful to clinical laboratories that participate in the validation testing of these products and be of general interest to laboratories in which rubella serological testing is carried out.

Part II (formerly NCCLS document I/LA7-P) is intended to create an awareness of the preanalytical factors related to rubella serology tests that may affect patient care. Sections 8 through 12 recommend procedures for collecting and handling specimens submitted for serological testing and describe the reporting and interpreting of test results.

[NCCLS. *Detection and Quantitation of Rubella IgG Antibody: Evaluation and Performance Criteria for Multiple Component Test Products, Specimen Handling, and Use of Test Products in the Clinical Laboratory; Approved Guideline*. NCCLS document I/LA6-A (ISBN 1-56238-335-3). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 1997.]

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Detection and Quantitation of Rubella IgG Antibody: Evaluation and Performance Criteria for Multiple Component Test Products, Specimen Handling, and Use of Test Products in the Clinical Laboratory; Approved Guideline

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Foreword

During the past 25 years, a variety of tests have been described for the detection and/or quantitation of rubella antibody. Several variations of the same basic test methodologies have been developed. In 1969, the Centers for Disease Control (CDC) (now the Centers for Disease Control and Prevention), Atlanta, Georgia, initiated a voluntary premarket evaluation and testing program for manufacturers of commercial in vitro rubella diagnostic products. Since 1982, the United States Food and Drug Administration (FDA) has classified rubella test kits as Class III devices. The first release of this document established standards for all multiple component products intended for the detection of rubella antibody. It defined the performance specifications required for these products. Products meeting these criteria are suitable for use by a laboratory in reporting results for medical management decisions. Scientific advancements and significant change in methods used for the detection of rubella antibody have led to revision of this document.

Development of this guideline was undertaken as a result of two suggestions: one, from the American Society for Microbiology, who suggested that NCCLS do a comprehensive study of rubella serological testing; the other, from the FDA Center for Devices and Radiological Health and several manufacturers of rubella testing products, who suggested that voluntary guidelines specifying a panel of reference preparations would be helpful to laboratories involved in rubella testing.

Initially, the two parts of this guideline were separate NCCLS consensus documents, I/LA6-T (*Evaluation and Performance Criteria for Multiple Component Test Products Intended for the Detection and Quantitation of Rubella IgG Antibody; Tentative Guideline*) and I/LA7-P (*Specimen Handling and Use of Rubella Serology Tests in the Clinical Laboratory; Proposed Guideline*). On the recommendation of the NCCLS Area Committee on Immunology and Ligand Assay, the guidelines have been combined in this approved level publication to provide to the clinical laboratory testing community a comprehensive resource focused on rubella serology.

In the previous edition of I/LA6-T, the subcommittee recommended the use of 10 IU/mL as the breakpoint for defining the probability of immune status and reaffirms this view in I/LA6-A. This edition of the guideline also incorporates a major revision in two respects. The product criteria outlines in greater detail the requirement to inform the user on the intended use of the test, and the sensitivity, specificity and reproducibility of the test. The second feature of this edition is expansion of sections on use of the test in diagnosis of subclinical and clinical rubella and congenital rubella. These sections should assist the user in interpretation of test results in unusual situations. Additionally, all the comments received on both previous editions have been considered in this revision, and responses to the comments are appended to this guideline.

The NCCLS Subcommittee on Rubella Serology recognizes the ongoing change in laboratory practices and welcomes constructive suggestions for improvement of this document.

Universal Precautions

Because it is often impossible to know which might be infectious, all patient blood specimens are to be treated with universal precautions. Guidelines for specimen handling are available from the U. S. Centers for Disease Control and Prevention [*MMWR* 1987;36(suppl 2S):2S-18S]. NCCLS document [M29](#)—*Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*, deals specifically with this issue.

Key Words

Rubella hemagglutination inhibition (HAI) antibody test, rubella serodiagnostic products, diagnostic product, product quality control, rubella product evaluation, rubella test kit, rubella reagents, performance criteria, clinical rubella, congenital rubella, immunity, IgG, IgM.

Detection and Quantitation of Rubella IgG Antibody: Evaluation and Performance Criteria for Multiple Component Test Products, Specimen Handling, and Use of Test Products in the Clinical Laboratory; Approved Guideline

Part I

1 Scope

Part I of this document establishes performance guidelines and criteria for evaluation of multiple component test products used to detect rubella antibody.

2 Nature and Structure of Rubella Virus

Rubella virus consists of at least three envelope glycoproteins: E1, E2a, E2b; a nucleocapsid-associated protein, C; and two nonstructural proteins.¹⁻⁵ The term "rubella antibody" describes immunoreactivity to some or all of the virus components. Individuals may respond immunologically in a diverse manner to the rubella virus. The reagent sets used for detection of antibody may react differently depending on the reactivity of the predominant antibody to the rubella peptide.

Serum samples from patients with various forms of rubella virus infection were tested for antibody to each of three viral structural proteins.^{4,6-8} In most sera, antibody to E1 protein was the predominant species. Sera from patients with congenital rubella syndrome, however, contained significantly more E2 antibody relative to E1 antibody than did sera from other rubella patients.

3 Status of Hemagglutination Inhibition Method

A standard rubella hemagglutination inhibition (HAI) antibody test was originally adopted by NCCLS in 1979 and by the World Health Organization (WHO)⁹ as the reference rubella antibody assay method. Previous studies have established a high correlation between rubella HAI and traditional rubella neutralization test results.¹⁰⁻¹¹

The HAI test lacks sensitivity at dilutions less than 1:8. Nonspecific inhibitors of hemagglutination cannot be reliably removed without giving rise to other nonspecific reactions.¹²

3.1 Use of HAI in Establishing the Calibration Standard

HAI serves as the reference method to establish a calibration standard for other rubella methods. Since relatively few or no laboratories in the United States use HAI for routine testing,¹³ an alternative for calibration is the use of the World Health Organization International Standard (WHO) for antirubella serum, human. The 3rd International Standard includes two other assay methods, the enzyme immunoassay and single radial hemolysis, in addition to HAI in evaluation of the reference preparation. (See Section 7.1.)

3.2 Relationship of HAI and Other Methods: Clinical Implications

Other methods including the commonly used enzyme immunoassay (EIA) and agglutination test, are capable of detecting levels of antibodies less than 1:8.¹⁴⁻¹⁶ Tests based on the principle of EIA provide a continuous assay of antibody and permit identification of very low levels of rubella antibody.¹³ Studies conducted by Grangeot-Keros and associates¹⁷ substantiate the increased sensitivity of EIA when compared with HAI tests in the detection of rubella-specific IgG. They used immuno-chromatographed recombinant proteins containing the three structural proteins of rubella virus which are immunologically and morphologically identical to wild type virions instead of viral lysates for antibody detection. Using methods other than HAI, immune individuals with specific but minimal detectable rubella antibodies have been identified.^{15,16,18-21} Most, if not all, individuals with minimal detectable levels of HAI antibody produce secondary-type antibody responses

upon revaccination and are likely to be immune.^{15,22-23} Revaccination of these people did not induce viremia and little or no rubella-specific IgM was produced.^{15,16} As more people with vaccine-induced immunity join the adult population, there is a need for more sensitive test methods to accurately assess serological status.¹⁸ The issue of the protective effect of very low antibody levels is further complicated by reports of rare instances of viremia in people with low antibody levels given rubella vaccine.^{22,23} (Refer to [Section 3.4](#) for information on the issue of defining the reference point for rubella tests.)

3.3 Reporting Results

In an attempt to standardize the system of reporting results, the subcommittee recommends reporting results of quantitative tests in International Units (IU) rather than in dilutions. The World Health Organization has released the third international standard for antirubella sera. Use of this standard, or subsequent standards provided by the World Health Organization, will help resolve uncertainty about the interpretation of antibody detected by tests more sensitive than HAI.²⁴ Refer to [Section 7.1](#) for additional information on the WHO standard.

3.4 Establishing a Reference Point to Define Immunity

A reference point for tests to detect antirubella IgG serves to establish the recommended lower level of specific IgG antibody protective against the disease and provide a threshold value for the test. Physicians use the threshold value in making clinical decisions. A reference point may originate from research data, study of large population groups, or by determination of the predictive value of a test and is not necessarily inclusive of every individual in a population.

The original recommendations from the Subcommittee on Rubella Serology (first published in the tentative edition of this guideline) established 1:8 or 15 IU/mL as the indicator of immunity.^{12,25-26} The early experiments which led to this definition of rubella immunity probably will never be repeated since the protective effect of an IgG antibody level of 1:8 or greater derived from study of

people following natural exposure to the wild virus. Newer, more sensitive techniques detect rubella specific IgG antibody at levels below 1:8. Numerous reports indicate that people who have been previously vaccinated with low or apparently absent levels of HAI antibody produce secondary-type responses upon revaccination and are clinically immune.^{14,27-34} Epidemiologic studies support these findings.^{18,19,35,36} Sporadic reports of viremia and/or reinfection among previously immunized persons with low antibody levels have been reported but cases of reinfection have also occurred in persons with antibody levels at or above the 15 IU/mL cutoff.³⁷⁻⁴⁰ Alterations of the complex immune system may explain variations in response among some individuals. Results of antibody tests for rubella, like other laboratory tests, must be evaluated in the context of the clinical setting.

Based on epidemiologic data, studies on vaccinated individuals with low levels of antibody and anecdotal reports, the subcommittee restates the recommendation of 10 IU/mL as the reference point for IgG antirubella antibodies.^{41,42} The effectiveness of rubella vaccination is well-documented and the 10 IU/mL antibody level is protective in the vast majority of persons.* Recognizing that sporadic and sometimes conflicting reports suggest a relationship between antibody level and protection against the rubella virus, the subcommittee did not advocate lowering the breakpoint below 10 IU/mL until additional data has been collected.

Although scientific data are the basis for selecting the reference point to define immunity, results of antibody tests for rubella, like other laboratory tests, must be evaluated in the context of the clinical setting.

3.5 Positive Sera

The subcommittee recommends reporting the level of rubella antibody in quantitative units as IU/mL. For qualitative or semiquantitative screening tests, the designation "positive"

* A gray zone exists when defining the reference point. For further discussion of the gray zone, see [Section 6](#).

identifies sera with IgG rubella antibody equal to or greater than (\geq) 10 IU/mL.

3.6 Negative Sera

The subcommittee recommends reporting results in quantitative units at levels at and above the equivalence zone. Antibody levels less than the lower limit of the equivalence zone may be reported in IU/mL, as "less than," or negative depending on the practice adopted in each laboratory. For qualitative and semi-quantitative screening tests, the designation "negative" identifies sera with antibody levels of less than ($<$) 10 IU/ml.

4 General Criteria for Product

In vitro diagnostic products are highly regulated in many countries. France, Germany, Japan, and the United States have strict regulations that apply to Rubella immunoassays.

(In vitro diagnostic products intended for commercial distribution in the United States are medical devices regulated by the Food and Drug Administration (FDA). Manufacturers of these products are subject to the general controls and other controls of the Food, Drug, and Cosmetic Act (FD&C Act or "the Act"). General controls of the Act are baseline requirements that apply to all medical device manufacturers. Unless specifically exempted, medical devices must be properly labeled and packaged, be cleared for marketing by the FDA, meet their labeling claims, and be manufactured under Good Manufacturing Practices (GMP) which is a mandated quality assurance program. For further information regarding FDA regulations, contact the Division of Small Manufacturers Assistance (DSMA) in the office of Health and Industry Programs, Center for Devices and Radiological Health. Telephone 800.638.2041 or FAX 301.443.8818.)

4.1 Quality Control Sera

Products (quantitative and screening tests) designed to test for rubella antibody should include a minimum of three control sera (one negative, one low-positive, and one high-positive) to be assayed in each test run. The control sera for quantitative tests should be expressed in international units and the low-positive should be in the range of 10 to 20 IU/mL. These controls must be distinct from calibrators used in the test procedure (see [Section 4.2](#)). For quantitative tests, the manufacturer should provide statistical data, i.e.,

mean and standard deviation for each lot number of control material.

The College of American Pathologists (CAP) provides a certified rubella reference material suitable as an alternate independent control. The reference material is intended for periodic use to validate the calibration procedure. The reference materials may be obtained from the College of American Pathologists, Standards and Reference Material Program, 3325 Waukegan Rd., Northfield, Illinois 60093-2750, Telephone 800.223.4040, Ext. 468.

4.2 Calibrators

Calibrators are required to substantiate the continued accuracy of the test method throughout the laboratory's reportable range for patient test results. The calibration materials should be appropriate for their method and, if possible, traceable to a reference method. Refer to the most current editions of NCCLS documents [I/LA18—Specifications for Immunological Testing for Infectious Diseases](#), and [C24— Internal Quality Control Testing: Principles and Definitions](#), for the appropriate definitions of calibrations and controls.

4.3 Description of the Specific Type and Class of Antibody

The rubella virus consists of major antigenic components associated with the viral envelope and the ribonucleoprotein core. The manufacturer must describe adequately the antigen and the specific type and class of antibody detected by the reagent set, i.e., whole virus vs. selected viral proteins. The impact of the nature of the antigen on the diagnosis of acute or congenital infection should be clearly stated.

The manufacturer should state whether or not the reagents are sensitive to early or late-appearing IgG antibodies, and the time it takes for a test to become positive following infection or exposure.

4.4 Labeling

Labeling should conform to in vitro diagnostic products labeling regulations. *(In the U.S. refer to 21 CFR 809.10 [Code of Federal*

Regulations.) The labeling of each component and of the entire unit should meet all federal requirements. On the label or package insert, indicate the intended use of the reagent set, e.g., determination of immune status, diagnosis of subclinical or clinical rubella, diagnosis of congenital rubella, and, if applicable, the sensitivity to early or late-appearing IgG antibodies (see Sections 4.3, 12.2.2, 12.3.1, and 12.4.2). Indicate the limitations of testing for the assay, e.g., not intended for use in diagnosis of congenital rubella.

5 General Performance Criteria

All components of the product should function as claimed by the manufacturer and produce the expected results with clinical specimens. For example, a manufacturer may claim that hemolysis, bilirubin, and lipemia do not interfere with the reactivity of a reagent set. Clinical studies should include a sufficient number of samples to verify the claim.

6 Specific Performance Criteria

Rubella sero-diagnostic products should demonstrate linearity with the WHO Standard over the range of the assay.

6.1 Sensitivity

Sensitivity is the ability of the assay to detect antirubella antibody in serum at the 10 IU/mL level (see Section 3.5). To be acceptable for in vitro diagnostic use, the assay must demonstrate sensitivity of 95% based on assay of 50 or more specimens with antibody levels exceeding 20 IU/mL. The assay must also demonstrate sensitivity of 95% based on assay of 50 specimens in the low range of 10-20 IU/mL. Samples should be drawn from the clinical population of interest including pregnant women, children, and adults.

The manufacturer may define a "gray zone" for the test but the limits must fall within the requirements for reproducibility (see Section 6.3). When a result in the series of tests for sensitivity falls in the "gray zone" the manufacturer should handle the results according to the recommendation it makes to users of the test, e.g., retest, redraw a specimen, etc. In the

calculation of sensitivity, equivocal results in the "gray zone" may be excluded but the manufacturer should indicate the frequency of this occurrence in their product labeling.

6.2 Specificity

Specificity is the ability of the assay to discern a rubella-negative serum with an antibody level less than 10 IU/mL from a rubella-positive serum (see Section 3.6). To be acceptable for in vitro diagnostic use, the assay should demonstrate specificity of 95% based on assay of 100 or more rubella-negative sera with antibody levels less than 10 IU/mL.

The manufacturer may define a "gray zone" for the test but the limits must fall within the requirements for reproducibility (see Section 6.3). When a result in the series of tests for specificity falls in the "gray zone," the manufacturer should handle the results according to the recommendations it makes to users of the test, e.g., retest, redraw a specimen, etc. In the calculation of specificity, equivocal results in the "gray zone" may be excluded but the manufacturer should indicate the frequency of this occurrence in their product labeling.

6.3 Reproducibility

Reproducibility is the ability of the product to show uniformity of antibody level in replicate testing of positive and negative sera based on within-run testing. The manufacturer should define the reproducibility of the test using three "manufactured" lots of sera. At the 10 IU/mL level, the 3rd standard deviation (SD) interval in repetitive tests (40 or more assays) should not exceed ± 3 IU/mL. For qualitative and semi-quantitative tests the manufacturer must define the "gray zone" in terms of IU/mL. For reagent sets intended to evaluate immune status only (i.e., reagent sets reporting test results as positive or negative) the product should demonstrate reproducibility of $\geq 95\%$ based on assays in duplicate of 40 or more rubella-positive sera with a titer in the range of 10 to 20 IU/mL and 40 or more negative sera.

For qualitative and semiquantitative assays, the manufacturer should indicate the imprecision of the method at three concentration levels—

negative, low positive, and positive. (See NCCLS document [EP10](#), *Preliminary Evaluation of Clinical Chemistry Methods*.)

6.4 Stability

The product should be shown to perform reliably throughout the term of its shelf-life as stated by the manufacturer and indicated in the product labeling. If a component is lyophilized, the manufacturer must indicate the shelf-life of the reconstituted component.

6.5 Suitability of Reagent Set for Diagnosis of Acute Infection or Reinfection

The manufacturer must indicate if the reagent set is suitable to determine a "significant change" in IgG antibody levels for the diagnosis of acute infection or reinfection ([Refer to Section 12](#) for additional information). The term "significant change" should be defined by the manufacturer and should include consideration of the assay imprecision. The manufacturer should concurrently recommend assay of specimens as good laboratory practice.

7 Product Evaluation

7.1 Calibration

The requirements for calibration include:

- (1) Manufacturers are required to use the World Health Organization International Standards for antirubella serum, human (see [Section 3.1](#)). The 3rd International Standard is currently available and may be procured by contacting:

International Laboratory
Statens Seruminstitut
Copenhagen, Denmark
Telephone: 45-32-68-31-50
Fax: 45-32-68-34-66
E-mail: gahansen@standard.ssi.dk

- (2) Calibration verification using the CDC low-titer rubella antibody standard with an assay value of 20 IU/mL. The calibration procedure should include testing with a ½ dilution of the standard. For procurement of the CDC reference preparation contact:

Centers for Disease Control and Prevention
NCID-DVRD-VEHB
Bldg. 7, Rm. 206, G-18
1600 Clifton Rd.
Atlanta, GA 30333

7.2 Statements of Signal Suppression or Interference

Manufacturers should state the concentration range, in IU/mL, of samples tested in clinical trials. Manufacturers should also indicate any signal suppression or interference (prozone or "hook" effect) observed during clinical or analytical studies.

7.3 Validation Testing

The reagents or kits, and any required special equipment are to be evaluated by at least two independent laboratories.

7.3.1 Evaluation Serum Panel(s)

Panels of known reactivity tested with at least two other commercially available assays as a reference must include an array of high positive, low positive, and negative specimens suitable to allow evaluation of test sensitivity, specificity, and reproducibility. Sera should be drawn from the clinical population of interest. ([See Section 6.1](#).) Clinical histories on rubella IgG immune individuals may be inadequate. The CDC provides a panel of coded sera for evaluation. The panel may be procured from the CDC at the address listed in [Section 7.1](#).

7.3.2 Reagents, Controls, and Equipment

All product components necessary to perform the test as defined by the manufacturer should be included in the evaluation. These components will be provided by the manufacturer for the evaluation.

7.3.3 Evaluation Design

The product will be evaluated by the designated laboratories using:

- (1) Evaluation serum panel(s);

(2) A panel of coded serum provided through the CDC;

(3) The CDC low-titer rubella antibody standard, including a ½ dilution of the standard. To procure the coded serum panel, contact the CDC at the address listed in [Section 7.1](#). The manufacturer will provide serum panels and standards for use by the designated laboratories.

7.3.4 Test Method

The test method used is that stated by the manufacturer in the product package insert.

7.3.5 Reports and Analyses of Validation Testing

The manufacturer should summarize results of these evaluations to validate product performance claims.

(All new *in vitro* diagnostic multiple component test products intended for the detection and quantitation of rubella antibody and headed for commercial distribution in the United States must undergo regulatory review by FDA before marketing. Contact FDA's Division of Small Manufacturers Assistance (DSMA) for technical assistance and regulatory guidance for complying with FDA requirements for medical devices.)

(It is recommended that manufacturers seek assistance to independently test these products and make data available to the FDA for review. This service may depend on availability of personnel and time at the CDC. Manufacturers should contact the CDC well in advance of the anticipated date for requesting this service.)

Part II

8 Introduction

Rubella tests are usually ordered for one of three purposes:

- (1) to establish immune status, natural or vaccine-induced immunity
- (2) to diagnose acute (recent or current) infection of clinical or subclinical rubella
- (3) to diagnose congenital rubella

Although the analytical aspects of the various tests for detection of antibodies to rubella are widely discussed, preanalytical factors and the

interpretation of testing have received scant attention. Misinterpretation of test results may lead to improper clinical decisions. "Black and white" interpretations are used even though certain questionable "gray" areas exist.

9 Scope

Part II of this guideline describes recommended procedures for collecting and handling specimens and discusses the reporting and the laboratory interpretation of test results. The laboratory interpretation of test results should be distinguished from the clinical interpretation. The physician should evaluate the laboratory interpretation of the data along with the clinical history and physical examination of the patient to determine immune status (particularly in critical cases of pregnancy) and in establishing the diagnosis of current infection or congenital infection with rubella virus. Part II should help various laboratory scientists perform and report the results of rubella serologic tests.

10 Clinical Background

10.1 Nature of Virus

Refer to [Section 2](#) for information on the nature of the virus and [Section 4.3](#) for the manufacturers requirement to describe the specific type and class of antibody detected by the reagent set.

10.2 Immunology²¹

10.2.1 Acute Infection

The incubation period of acute rubella is 14 to 21 days. Initially, antibodies in both the IgG and IgM classes can be detected. Immunoglobulin M antibodies generally do not persist beyond 5 to 6 weeks after the onset of illness, whereas IgG antibodies usually persist for the lifetime of the patient.

A number of methods are currently used for detecting rubella antibodies. The HAI test detects early IgM and later-appearing IgG antibodies. The passive hemagglutination test is sensitive to late-appearing IgG antibodies, which may not be detectable until weeks after onset of a rubella infection. Other test systems, such as

enzyme immunoassay and latex agglutination, have immunoglobulin specificities controlled by the antiglobulin conjugate used in the indirect assay. Refer to Section 4.3 for additional information on the requirement of the manufacturer to specify the characteristics of the antigen in the package insert.

10.2.2 Serological Pattern After Vaccination

Immunization with the attenuated rubella virus vaccine induces the formation of rubella-specific IgM and IgG antibodies similar to those observed with natural infections. The antibody titers are usually lower than those observed in natural infections and the complement-fixing antibodies are definitely found in lower titer.

10.3 Complications

Several investigators have established the relationship between congenital anomalies and maternal rubella infections. Infection with rubella virus during childhood or adulthood is usually a benign self-limited disease. However, the rubella infection may be complicated by transient arthralgia or arthritis. Rare, infrequent complications such as encephalitis and/or thrombocytopenia may occur.

Infection of the fetus during the first trimester and, to a lesser degree, the second and third trimesters may result in congenital rubella with birth defects such as neurosensory deafness, heart anomalies, cataracts, and retardation of growth. In neonates with congenital rubella infection, the virus may persist for 6 to 9 months after birth.

11 Specimen Collecting and Handling

11.1 Specimen

The vast majority of laboratories rely on reagents in prepackaged diagnostic kits in testing for antirubella antibodies. The manufacturers of diagnostic kits, by federal regulation, must establish the specimen type to be used in testing. While testing with serum prevails, users are advised to follow the manufacturer's package insert. For some reagent kits, plasma may be an acceptable specimen.

NCCLS documents H4, *Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture* and H18, *Procedures for the Handling and Processing of Blood Specimens*, provide detailed information on specimen collection and handling. Collect and handle the specimen, usually serum, aseptically. Since microbial growth may interfere with the accuracy of testing, use of aseptically collected specimens is particularly important when testing is delayed and/or specimens are stored for testing at a later date.

(1) Aseptically remove the specimen, usually serum, and place it in sterile, capped tubes labeled with the specimen submission number, patient's name, and other required data. Sterile serum separators are a suitable means of transferring sterile serum specimens for testing and/or storage.

(2) Use aseptic technique and equipment when removing a portion of the specimen, usually serum, for testing.

11.2 Data Provided to the Laboratory

The majority of tests are performed to determine the need for vaccination in people without a history of exposure and/or without related clinical findings. In these instances, patient information follows the requirements for other laboratory tests.

For the proper assistance to the physician in test selection and interpretation of test results when the clinical setting or findings suggest rubella exposure and/or infection, additional patient information includes:

- immunization history for rubella,
- date of onset of symptoms,
- major clinical findings, and
- purpose for which specimen is submitted, e.g., immune status evaluation, congenital infection, serologic diagnosis of infection.

11.3 Storing Specimens

(1) Separate plasma or serum from blood cells as soon as practical. Use sterile technique if samples are to be stored longer than 48 hours. A preservative such as sodium azide should be added if samples are to be stored at refrigerator temperature for greater than 48 hours. Serum or plasma may be stored at 2-8° C for up to one week if separation has been performed as listed above with a preservative added (written communication, A.M. Johnson, M.D., December 1996).

(2) For long term frozen storage, tightly sealed freezer tubes with minimal dead (air) space should be used. Immunoglobulins are stable at -20° C for at least a year if these recommendations are followed. For longer term storage, -70° C storage is recommended. To avoid repeated freeze-thawing cycles of samples likely to be used repeatedly, multiple small aliquots in separate small tubes are recommended (written communication, A.M. Johnson, M.D., December 1996).

(3) We recommend holding specimens for at least one year. This is particularly important for later follow-up studies of pregnant women who are inadvertently exposed to rubella virus.

NCCLS document [H18](#)— *Procedures for the Handling and Processing of Blood Specimens*, provides detailed information on specimen storage.

11.4 Filing System

Establish a filing system of specimen storage for easy specimen retrieval at a later date.

12 Clinical Use of the Test(s)

12.1 Legally Marketed Commercial Test Kits

The WHO International Rubella Standard is the reference for all reagent sets.

(In the United States all reagent sets must be cleared for marketing using the FDA's premarket notification process; Refer to Sections 4.0 and 7.3. The subcommittee recommends use of reagent sets that have included an evaluation by the CDC.)

In the selection of a reagent set, refer to [sections 2](#) and [4.3](#) for information on the nature and structure of rubella virus and the manufacturers requirements to describe the specific type and class of antibody detected by the reagent set. Note that in testing for congenital rubella syndrome, the reagents must demonstrate the ability to detect E2 antibody.

Refer to Section 6.5 for the requirement of the manufacturer to indicate if the reagent set is suitable to determine a "significant change" in IgG rubella antibody level for the diagnosis of acute infection or reinfection.

12.2 Establishing Immune Status

12.2.1 Specimen

To evaluate the immune status after a past infection or from immunization, test a single specimen, usually serum.

To assess the immune status of a pregnant woman exposed to rubella virus, obtain the specimen within ten days after exposure and test for IgG and IgM antibodies. The antibody from previous infection is of IgG class.

12.2.2 Appropriate Tests

Qualitative, semiquantitative or quantitative tests are acceptable in the routine assessment of the immune status.

12.2.3 Reporting Results

Follow the package insert accompanying reagent sets for the manufacturer's recommendation regarding the threshold or "cut-off" point for defining immunity and the units for reporting results. In reporting the results, indicate the generic and proprietary name of the assay. These results derived by different assays are not necessarily interchangeable. Provide the physician with the threshold value to define the immune status or interpret the test result, particularly when qualitative tests are used. For quantitative tests, report results in IU/mL.

(1) Report results in quantitative units or as "positive" (antibody at or greater than 10 IU/mL) and "negative."

(2) If the manufacturer defines a borderline zone around the threshold value of the test, marginal results should be annotated. Laboratories are advised to establish a definitive procedure for dealing with the issue of "borderline" results, i.e., additional testing by another method, additional specimen for assay, physician notification, etc. The manufacturer may provide recommendations for dealing with this issue. Refer to Section 6.3 for requirements of the manufacturer to define the reproducibility of the test and Sections 3.5 and 3.6 for additional information on reporting results.

12.2.4 Interpreting Results⁴³

(1) In instances in which there is no history of exposure or possible infection, the presence of antibody at or above the threshold level is evidence of previous exposure/infection with rubella virus at an undetermined time, either as a result of immunization or natural infection.

(2) The presence of IgG antibody in a specimen from a pregnant woman taken less than ten days from exposure to rubella virus is presumptive evidence of a previous infection at some time. In instances of exposure to rubella virus, the potential of reinfection should be considered on an individual basis. Refer to item (3) below for additional guidelines on the interpretation of test results, especially in pregnant women.

(3) Diagnosis of reinfection: Rare anecdotal reports occurring in locations outside of the United States cite instances of viremia without clinical symptoms, in vaccinated persons exposed to the live rubella virus.⁴⁴⁻⁴⁶ Rare reports document symptomatic rubella reinfection in vaccinated individuals or those with naturally acquired clinical rubella.^{37,47} In these unusual circumstances, additional testing may show an IgM response or an accelerated IgG response without formation of rubella-specific IgM.³⁷⁻³⁹ Each case must be considered individually in regard to the interpretation of test results.

The criteria for diagnosis of reinfection include^{39,48}:

- a specific but unusually low level IgM response, and/or

- a significant rise in (IgG) antibody concentration.

In cases in which reinfection is considered several weeks after possible exposure and the rise in IgG or IgM antibody may be missed, the diagnosis of reinfection must rely on evidence of pre-existing rubella antibody by retesting stored specimens or laboratory reports of two or more previous positive rubella antibody tests. A documented history of rubella vaccination followed by one positive test is acceptable evidence of pre-existing antibody because the possibility of vaccine failure combined with laboratory error is remote.^{39,48} Refer to the Section 12.3 for recommendations on appropriate tests for diagnosis.

12.3 Diagnosis of Subclinical or Clinical Rubella⁴³

12.3.1 Appropriate Tests to Use

Rubella-specific IgM antibody tests are preferred for confirmation of subclinical or clinical rubella.

Testing for rubella IgG antibodies has changed dramatically with the introduction of newer methods and previous recommendations on use of acute and convalescent-phase specimens to detect a change in antibody titer may not be applicable. Follow the package insert recommendations in selection of the test for diagnosis of asymptomatic or clinical rubella. For some quantitative IgG rubella tests, the standard curves do not show a linear relationship between the optical readings and antibody levels and attaching clinical significance to changes in quantitative values in these instances is less than desirable.

For the diagnosis of subclinical and clinical rubella, test selection and interpretation must be made on an individual basis. The selection includes:

- Use of an IgM antibody test to detect recent infection. Rheumatoid factor may potentially cross react with IgM tests and the factor must be removed unless the manufacturer provides for its removal in the assay protocol. Since IgM tests may be subject to false positive results, confirmation by alternate methods is

recommended, i.e., significant increase in antirubella IgG, detection of viral protein, or detection of viral nucleic acid.

- Use of a quantitative IgG test to detect a significant difference in antibody concentration between the acute- and convalescent-phase samples when testing the pair in the same run to avoid differences in between-run sensitivity. Follow the package insert recommendations when selecting a quantitative IgG test for diagnosis of subclinical and clinical acute rubella. [Refer to Section 6.5.](#)

12.3.2 Specimens

Selection of specimens relates to the test selected for diagnosis of subclinical or clinical rubella.

- (1) Acute- and convalescent-phase serum samples are required to demonstrate a significant change in antibody concentration during the course of the infection. Obtain the acute-phase sample early in the infection, preferably less than seven days from onset of symptoms, and the convalescent-phase sample one to two weeks after the first sample, but not earlier than ten days after onset of symptoms.
- (2) During this period, and depending on the test system used, antibody of both the IgM and IgG classes will be detected. Some solid-phase assays are specific for either IgG or IgM antibody. IgG antibody concentration rises during the first two weeks after onset of the rash and remains elevated indefinitely.
- (3) A single specimen may be sufficient to determine IgM antibody only. Obtain this specimen early (within two weeks) after the onset of the rash. IgM antibody concentration rises during the first two weeks after the onset of the rash and usually declines to undetectable levels in two to three months or less.

12.3.3 Reporting Results

The system for reporting results relates to the test used for assay and the manufacturer's recommendations. The report of a qualitative IgM antibody test should be either "positive for IgM antibody" (IgM antibody present) or

"negative for IgM antibody" (IgM antibody not detected).

For quantitative IgG tests, report results in quantitative units as IU/mL. Indicate the generic and proprietary name of the test. (The laboratory should retain detailed information on reagent set identification.)

A laboratory interpretation should accompany the report. NCCLS document [I/LA18—Specifications for Immunological Testing for Infectious Diseases](#), provides detailed information on reporting results.

12.3.4 Interpreting Results

- (1) The presence of antirubella-specific IgM antibody is presumptive evidence of a current or recent infection.
- (2) For IgG antirubella tests used to support the results of IgM testing, follow the manufacturer's recommendation for interpretation of a significant change in antibody concentration between acute- and convalescent-phase specimens.
- (3) If a traditional serial dilution test is used, a four-fold or greater rise in antibody titer between the acute- and convalescent phase specimens is evidence of a current or recent infection with rubella virus.

12.4 Diagnosis of Congenital Rubella [43,49,50,51](#)

12.4.1 Specimen

The criteria for the laboratory diagnosis of congenital rubella include:

- Rubella virus isolation from nasopharyngeal, urine, cerebrospinal fluid specimens
- Testing blood for the presence of rubella specific IgM antibodies
- Persistence of rubella-specific IgG antibody at quantitative levels above and beyond that expected from passive transfer of maternal antibody ([See Section 12.4.4](#))

- Western blot analysis of all three structural proteins.

12.4.2 Appropriate Tests to Use

IgM antibody tests using cord blood is the preferred serologic method for the diagnosis of congenital rubella. Rheumatoid factor may potentially cross-react with IgM tests and must be removed unless the manufacturer provides for its removal in the assay protocol. Confirmation by an alternate method is recommended.

Virus isolation techniques are the preferred alternate method.

Indirect tests for rubella specific IgG antibody may be used in instances in which the diagnosis is considered at an interval after birth when IgM testing or virus isolation techniques are not applicable.

For the diagnosis of congenital rubella, the IgG test must detect the whole virus as compared to tests which detect antibodies to selected viral proteins. Sera from patients with congenital rubella have increased ratios of antibody E2 relative to E1 antibody.^{4,6-8,51} Tests that detect predominantly E1 antibody should not be used. Refer to Sections 2 and 4.3 for additional information on the nature of the rubella virus and the requirement of the manufacturer to indicate the nature of the antigens in their assays.

12.4.3 Reporting Results

Report results as described for clinical infection.

12.4.4 Interpreting Results^{43,49,50}

Because maternal IgM antibody does not pass the placental barrier, IgM antibody in cord or neonatal blood has been produced by the fetus, and its presence in the blood is presumptive evidence of congenital infection. Additional testing may be required for confirmation. However, the absence of IgM antibody does not rule out congenital infection. Contamination of cord blood with maternal blood negates the use of this specimen for testing.

If infection of the fetus occurs in the first trimester of pregnancy, 90% of infected babies will have detected circulating IgM antibody; if

infection occurs very late in pregnancy, only about 11% of infected babies will have circulating IgM antibody.²⁸

Because IgG antibody passes the placental barrier, most IgG antibody in cord or neonatal blood will be of maternal origin. If the IgG antibody was from the mother, the concentration will have significantly declined in the baby's sixth month sample and may be below detectable levels. If it was produced as a result of congenital infection, it will have persisted.

12.5 Record Keeping

Maintain a dated and numbered record sheet for each run. Enter all pertinent information related to the run on the record sheet, such as:

- (1) test used
- (2) lot number of reagents and concentrations used
- (3) results of antigen titration (if applicable)
- (4) results of positive and negative control sera
- (5) standards or calibrators.

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Summary of Comments and Subcommittee Responses

I/LA6-T: *Evaluation and Performance Criteria for Multiple Component Test Products Intended for the Detection and Quantitation of Rubella Antibodies; Tentative Guideline*

General

1. The guideline should include the correlation of latex to EIA and the reason there may be occasional differences.
 - **This NCCLS guideline is not intended to identify differences between methods. The methods may have unique immunoglobulin specificities controlled by the antiglobulin conjugate.**
2. Would it not be more in keeping with modern usage to report results in kIU/L rather than IU/mL?
 - **The subcommittee recognizes that kIU/L units has increased in popularity, especially in European countries. However, the subcommittee believes, based on the experiences of its members, that reporting IU/mL remains an appropriate alternative.**

Section 3.4

3. Using a fluid sample handler system we occasionally find considerable variation around the 15 IU/mL cut-off for rubella. I am concerned that 10 IU is too low, requiring ideal conditions. Can a 12 to 13 IU/mL cut-off be considered?
 - **Each laboratory must consider the variation in testing when establishing the reference point of "positive" versus "negative." The subcommittee has recommended to manufacturers the need for statistical data describing the expected variation in testing, especially around the breakpoint (see Section 6.3). The subcommittee recognized the existence of a borderline zone around the breakpoint value of any test and marginal results should be properly annotated (refer to Section 12.2.3).**
4. Reference #19 uses only an EIA of 10 IU/mL but a PHA of 7.5 IU/mL and an HAI of 5.0 IU/mL. Could not an EIA also go down to 5 or 7.5 IU/mL? If the loss of specificity was the reason 10 IU/mL were selected as the cut-off value it should have been stated more clearly in the NCCLS document. 10 IU/mL is not the obvious answer I read from reference #17. Also reference #25 is a letter to the editor that doesn't say very much new i.e., it repeats that some patients, seronegative with an HAI titer of < 1:8, are indeed immune based on the earlier booster studies.
 - **Sections 3.2 and 3.4 address the process of selecting 10 IU/mL as the reference point for routine rubella testing. Factors to consider include the variation in testing, especially in the range of the reference point and the risk of false positive results if 7.5 IU/mL or 5.0 IU/mL were selected.**
5. The meaning of the sentence, "The majority of seropositive persons are detected with 10 IU/mL as the indicator of immune status." is not clear by itself. No references are given for this sentence which would also be true if 5, 7.5, 12.0, 12.5, or 15.0 were substituted for 10 IU/mL. Again if the reason for not wanting to go to 5 or 7.5 IU/mL is concerned about specificity this should be stated more clearly in the document.
 - **Refer to the response for Comment 4.**

6. Since the early booster or vaccination studies were done before 5.0 - 12.5 IU/mL quantitative information was available, 10.0 IU/mL as an arbitrary cut of is again raised. Also there is no data available to scientifically select a cut-off value below 15 IU/mL at the present time. Particular challenge studies on volunteers with well defined quantitative seronegative Rubella values (below 15 IU/mL) have not been performed.
- **Sections 3.2 and 3.4 and the response to Comment 4 address the process of selecting 10 IU/mL as the reference point.**
7. We are wondering where exactly the committee stands on the recommendation of 10 IU/mL as the indicator of immune response. Our company takes the position that selecting 15 IU/mL is a more safe breakpoint for protective immunity, because considering pregnant women with antibody level of 10–15 IU/mL as being non-immune would result in appropriate behavior (avoid contact with rubella infected individuals) and appropriate vaccination strategy (vaccinate post pregnancy). As rubella vaccines are inexpensive more vaccinations by choosing a higher cut off are preferable to the risk of overestimating anti-rubella titer (levels below 10 IU/mL overestimated with test result of 10 IU/mL up to 15 IU/mL) or false positive anti-rubella IgG result (no rubella antibodies with test result > IU/mL due to unspecific reactivity).

In addition, recommending a cut off in IU/mL is a critical issue because different manufacturer's reagents do not necessarily correlate quantitatively. The papers of Dimech et al: Multicenter Evaluation of Five Commercial Rubella Virus Immunoglobulin G kits which report in International Units per Milliliter. JCM 30, 633–641 (1992) and Pustowitz et al.: Evaluation of recombinant rubella-like particles in a commercial immunoassay for the detection of anti-rubella IgG, Clinical and Diagnostic Virology 5(1996):13–20, discusses the issues of different calibration of rubella IgG reagents leading to miscorrelation between assays.

- **In this document, the subcommittee restates the recommendation of 10 IU/mL as the reference point for use by the clinical laboratory to define immunity against rubella. Epidemiologic data, studies on vaccinated individuals with low levels of antibody, and anecdotal reports provide adequate evidence that the 10 IU/mL antibody level is protective in the vast majority of individuals. Keep in mind that immunity in a given patient is a clinical decision and the results of anti-rubella tests, like other tests, must be evaluated in the context of the clinical setting. Sporadic reports of viremia and/or reinfection among previously immunized individuals with low antibody levels have been reported but reinfection has also occurred in persons with titers at or above the 15 IU/mL breakpoint. Despite the occasional occurrence of rubella reinfection in individuals with low titers, the theoretical risk is small especially when compared with the significantly greater risk in individuals who have not been vaccinated. Refer to reference 40 for more information.**

I/LA6-A includes the recommendation that the reproducibility (3 standard deviation interval) of anti-rubella tests should not exceed 3 IU/mL at the 10 IU/mL level. This recommendation is significant because, for the first time, reproducibility limits are defined and accrediting agencies can establish firm guidelines when evaluating performance of this test.

The Subcommittee on Rubella Serology has taken the first step leading to improved testing. Other problems remain and should receive attention. Manufacturers cite difficulty in use of the WHO standard and the calibration procedure lacks uniformity. Results derived by various commercially prepared reagent sets are not interchangeable.

The subcommittee welcomes additional dialogue on these issues and appreciates recommendations to improve the quality of this laboratory test.

8. The reference point does not establish the protective level; that can be determined only by following inflection rates at various levels.
- **In laboratory medicine it is customary to establish decision levels to guide physicians in medical diagnosis. The data currently available indicate that 10 IU/mL is a suitable indicator. A recent report using a new EIA test and recombinant rubella-like particles showed that borderline values of between 10 and 15 IU/mL were truly positive by Western blot analysis.¹⁷**

Section 4.1

9. The Guideline requires that “products... should include a minimum of three control sera (one negative, one low-positive, and one high-positive) to be assayed in each test run.” There is current commercial product which performs acceptably that calls for only two control sera (one negative, one low-positive). In addition, that product doesn’t supply the control sera as part of the product package. We believe the guidance in this respect is overly prescriptive.
- **The items cited are recommendations reflecting the professional experience, judgement, and agreement of the subcommittee and are not intended to be prescriptive. The subcommittee’s intent is that they serve as a conservative series of recommendations that do not preclude the use of alternative limits or ranges when appropriate, based on a product’s design or a laboratory’s quality control procedures.**

Section 4.3

10. What in reality is meant by “normal antigen control?” The impression in the guideline suggests that it is substrate which, like non-cultured rubella medium extract should be used as a “blank” for each sample to prove that the sample in general can be analyzed with the method. Is this an acceptable interpretation? How do we get such a control? Please explain the use of the control in detail.
- **A normal antigen control is not required with the new generation of rubella tests.**
11. How many parallel studies must be used when performing the reproducibility test?
- **Section 6.3 recommends repetitive testing of 40 or more sera. Three sites should be chosen to evaluate the product. Reproducibility should be performed on one lot of the product with at least 2 runs of testing per day for 20 working days. The runs should be separated by at least 2 hours. Within run, between run, and total reproducibility should be calculated using NCCLS guidelines.**
12. How many manufacturing lots shall be tested for performance criteria at the minimum?
- **At least three lots of product should be evaluated during a clinical evaluation to evaluate lot-to-lot difference.**
13. In how many different laboratories must the performance characteristics be verified? Is it possible to use either a research and development or quality assurance laboratory of our own as one of the selected laboratories?
- **Three different sites should be chosen to evaluate a test. Ideally, they should be located in different geographic locations to provide some heterogeneity in population. One of the sites may be a research laboratory of the manufacturer but the evaluation provided by this laboratory should not exceed one-third of the total evaluation data.**

Section 6.1

14. As indicators of sensitivity, the low positive sera are of real significance. High positive sera likely only detect false negative reactivity resulting from negative prozone effects, gross insensitivity, or transcription errors. Including so many of them (150) in the panel is a waste of reagents while they give an exaggerated sense of sensitivity.
- **Section 6.1 of I/LA6-A has been revised and greater emphasis placed on sensitivity in the range of 10-20 IU/mL.**

Section 6.2

15. This is an absolute minimum standard. Realistically, 5 false positives in 100 tests is hardly an acceptable standard unless they come from sera with borderline levels of antibody. Considering the possible consequences of infection and CRS in the absence of true rubella antibody, no company can afford to accept the legal liability of calling a result from a truly susceptible individual as positive.
- **All tests for antibodies against rubella show variation at the cutoff level of 10 IU/mL. False position or false negative results may occur. For this reason, the guideline advises manufacturers to establish a borderline range to indicate an antibody level in the equivocal zone. Repeat testing or immunization may be advisable. The clinical situation also dictates the response of the physician. If the patient is not pregnant, repeat immunization may be advisable. In a pregnant patient, additional testing or observation may be the prudent approach.**

Section 6.4

16. This section is unclear as to its meaning.
- **This section is intended to underscore the importance of documentation from the manufacturer on the stability of the reagents. In addition, laboratories using the reagents may monitor stability by using multiple controls. This has been clarified in Section 6.4.**

Section 7.0

17. This section should reference the NCCLS document RS13-P, *Rubella Antibody; Proposed Summary of Methods and Materials Credentialed by the NRSCL (National Reference System for the Clinical Laboratory) Council*.
- **This reference has been added to the list of NCCLS Related Publications.**

Summary of Comments and Subcommittee Responses

I/LA7-P: *Specimen Handling and Use of Rubella Serology Tests in the Clinical Laboratory; Proposed Guideline*

General Comments

1. I'd be inclined to caution against using IgM tests as part of a torch screen or indiscriminately on "high titer" specimens. Each of these approaches is used and both are likely to yield more false positives than true positives. The IgM test should be reserved for patients with a strong clinical history for rubella or with highly suggestive clinical findings.
- **Sections 12.2.4 and 12.3.1 of I/LA6-A utilize rubella IgM testing for diagnosis of subclinical or clinical rubella and rubella reinfection. The document does not deal with the issue of rubella IgM testing as part of the TORCH screen.**
2. More discussion on immune status and single-serum tests should be included.
- **This document provides an overview of rubella testing and is not intended to identify differences between various methods.**
3. The scope of the project should be expanded to define the difference in principles between reference methods (HAF) and screening methods (PHA, complement fixation).
- **Refer to response to Comment 2.**
4. It would be helpful if information on the how to remove rheumatoid factor was included.
- **The Area Committee on Immunology and Ligand Assay has considered establishing guidelines for rubella IgM reagents and testing procedures. The interest in IgM testing is very limited and therefore no project has been authorized to date.**
5. Methods and principles as stated are very vague. I would like the guideline to go into greater detail about the isolation of IgM.
- **Refer to response to Comment 4.**
6. The committee could include some statement about there liability and sensitivity of the various methods used, such as a comparison of IFA and ELISA and recommendations on the use of commercial systems.
- **Sections 12.2.4 and 12.3.1 of I/LA6-A utilize rubella IgM testing for diagnosis of subclinical or clinical rubella and rubella reinfection. The document does not deal with the issue of rubella IgM testing as part of the TORCH screen.**
7. It is recommended that the "limited value" be defined in this document.
- **The term "limited value" does not routinely appear in the medical literature and has not been used by the subcommittee. Its definition is uncertain to the subcommittee.**

Section 12.0

8. In Section 2.0 (now Section 9.0), perhaps the first paragraph should be deleted. It appears to be redundant in that all of the information in the first paragraph is also contained in the second paragraph. The second paragraph is far more complete and more to the point.
- **Section 9.0 has been modified to eliminate the redundant information.**

Section 4.1.1

9. The use of a sterile serum separator tube should be considered as an alternative to aseptic technique mentioned in this section (now Section 11.1).
- **Section 11.1 has been revised to include use of sterile serum separators.**

Section 4.2

10. Submission Slip Items 4-7 (now Section 11.2) are extremely difficult to obtain, especially on an outpatient basis. Patient histories are difficult to obtain. We are lucky if we get the name.
- **Section 11.2 has been revised to reflect the type of information provided with the specimen. Medical staff education is recommended in situations in which rubella exposure or infection is under consideration.**
11. Date and time should be included on the submission slip.
- **The subcommittee agrees that date and time are standard information, especially with the use of computerized systems.**

Section 4.3.1

12. Eliminate "if kept sterile" and replace it with "up to one week." (This now appears in Section 11.3.)
- **Storage of specimens for one year presents a problem in some laboratories. The recommendation for one year storage is intended to allow for retesting of pregnant women who may be exposed to the rubella virus. This issue is particularly important for the diagnosis of reinfection. See Section 12.2.4 of I/LA6-A.**

Section 4.3.2

13. Change "Store nonsterile specimens" to "To store specimens longer than one week, freeze." (This now appears in Section 11.3.2)
- **Section 11.3 of I/LA6-A has been revised.**

Section 4.3.3

14. Regarding frost-free freezers (now in Section 11.3), I am not certain that what is stated is so; see the introduction to CAP publication PPSH Hematology fascicle for a nice discussion.
- **The recommendation regarding frost-free freezers has been removed from this revision of the document.**

15. I question the recommendation of holding the specimen for at least one year (now mentioned in Section 11.3) for the following reasons:
1. The test results would be part of the medical and laboratory record.
 2. If there is a conflict between the past and current results, you cannot retrospectively manage the patient.
 3. The cost of maintaining the specimen, the relevant demographic information and a retrieval system would be enormous.
- **Refer to Comment 12 and its response.**
16. Storing specimens for one year presents a real hardship for hospital labs with little storage capacity.
- **Refer to Comment 12 and its response.**

Section 5.1

17. This section (now Section 12.1) indicates that the HAI is the validating test against which other tests should be measured. Consider the following:
1. MMWR 30(4): 38, 1981 "There are now more sensitive measures than the HI test to determine rubella immunity."
 2. CDC's summary analysis for Public Health Immunology 1984-1991 "Although the HI test is still considered the acceptable "standard" in the field of rubella testing, an increasing amount of evidence is being collected to challenge that traditional position."
 3. Similar challenges have appeared in the scientific literature such as those reported in:
 - a. JAMA 251: 1974, 1984
 - b. J. Infect. Dis. 149: 459, 1984
 - c. Numerous other scientific papers
- **Despite its limitations, the HAI test is the primary standard used for evaluation of the WHO preparation. HAI served as the initial standard in extensive clinical studies. Eventually, a more sensitive method probably will replace HAI. In current practice, the EIA is used as the reference assay to establish cut-off values to define the immune state. The method of assay is not the issue of immediate concern. Before discarding the HAI, attention should focus on two issues: (1) various methods, including the more sensitive assays, show discordant results, and (2) standardization of the procedure for reconstitution and storage of the WHO standard. When these issues have been resolved, transition to another standard would be appropriate.**
18. In last paragraph (of the new Section 12.1), "run the new test for a time in parallel" is ambiguous. It should read "Use control specimens with negative, low titer (ex. 1:8) and high titer (positive). When switching to a new test or lot of reagents evaluate performance on control specimens and 20 patient specimens. If concordant results are found, proceed with the new test or lot of reagents. Keep statistics on patient findings to monitor the incidence of negative and positive findings including the various titers.
- **Section 12.1 has been revised to address this comment.**

Section 5.2.1.1

19. From the first sentence (now in Section 12.2.1) remove the word "previous" from "previous past infection."

- **Section 12.2.1 has been modified as recommended.**

Section 5.2.3.1

20. Remove the words "reactive" and "nonreactive" [now in Section 12.2.3(1)]. The tests are designed to detect antibody and test result should be reported exactly as that — antibody, in this case, "rubella antibody detected." Qualitative test results should be reported as immune/not immune.

- **Section 12.2.3 has been revised accordingly.**

Section 5.2.3.3

21. This section [now 12.2.3(3)], states, "For an immune status report, a titer of 8 is the minimal recommended titer for a report of "reactive" (antibody present)." Using the titer of 1:8 appears to be in conflict with:

1. MMWR 30(4): 38, 1981 "Any detectable titer, even if very low, protects against subsequent viremic infection ..."
2. Report published in J. Infect. Dis. 149: 459, 1984
3. Data contained in the CDC Premarket Evaluation Program Support No. 13. in which the Rubascan is approved for testing for immune status. Rubascan uses a 1:1 titer to indicate the presence of antibody (immunity). Furthermore, this document (I/LA7-P Section 5.1) recommends using kits that passed the product evaluation.

- **Refer to response to Comment 4.**

Section 5.2.3.4

22. The recommendation [now in Section 12.2.3(4)] is made that marginal results should be reported as such. Perhaps some comment should be made about the need for retesting these marginal or "grey area" specimens.

- **Section 12.2.3 of I/LA6-A has been revised to address this comment.**

Section 5.2.4

23. This section on interpretation of results (now Section 12.2.4) should be expanded. The critical specimens are not considered in this document such as a pregnant woman whose first serum is tested more than 10 days after exposure. The type of testing, (i.e., IgG or IgM) along with the time interval of testing and interpretation should be included in this document.

- **Section 12.2.4 has been revised to address this comment.**

Section 5.3.2

24. This section (now 12.3.2) should indicate when a CF, HAI, etc. is indicated or not clinically indicated. This omission is a serious deficiency.
- **This NCCLS guideline is not intended to identify differences between methods. The methods may have unique immunoglobulin specificities controlled by the antiglobulin conjugate.**

Section 5.3.2.1

25. As I am sure you are aware, the numerous tests which are used have a variety of ways in which titers and ratios are determined. You have a difficult challenge in trying to write a paragraph describing appropriate tests to use (now Section 12.3.2).
- **The trend in manufactured reagent sets is to use IU/mL and the document has been written to reflect the new direction in reporting results.**

Section 5.3.2.2

26. It should be noted that the reagents for IgM antibody testing are currently quite expensive.
- **In developing standards and guidelines, NCCLS committees are concerned with cost effectiveness, but, NCCLS documents generally do not deal with financial considerations.**
27. Comments are made (now in Section 12.3.2) about the occurrence of rheumatoid factor and the fact that this RF factor can give a false positive result in specimens which are being tested for IgM antibody. The statement is made that the rheumatoid factor must first be removed before testing for rubella-specific IgM antibody. A reference which supports these comments and which defines the treatments which are necessary for removal of the rheumatoid factor should be added to this document.
- **The Area Committee on Immunology and Ligand Assay has considered establishing guidelines for rubella IgM reagents and testing procedures. The interest in IgM testing is very limited and therefore no project has been authorized to date.**
28. This section (now Section 12.3.2) should include information on which commercial tests are available and their relative sensitivity and specificity.
- **This NCCLS guideline is not intended to identify differences between methods. The methods may have unique immunoglobulin specificities controlled by the antiglobulin conjugate.**

Section 5.3.3.4

29. I would suggest removing "reactive for IgM antibody" and "nonreactive for IgM antibody" from this section [now Section 12.3.3(4)].
- **Section 12.3.3 includes alternate terms to describe the results of testing, i.e., "IgM antibody present, IgM antibody not detected."**
30. Include information on IgM isolation tests available and their relative sensitivity and clinical relevance.
- **See response to Comment 27.**

Section 5.5.1

31. Define what is meant by "Conditions of the test" (now in Section 12.3).

- **Section 12.3 has been revised to address this comment.**

Related NCCLS Publications[†]

- DI1-A2** **Glossary and Guidelines for Immunodiagnostic Procedures, Reagents, and Reference Materials—Second Edition; Approved Guideline (1992).** Provides common terminology and basic methodology for users and manufacturers of immunodiagnostic systems.
- DI2-A** **Immunoprecipitin Assays: Procedures for Evaluating the Performance of Materials; Approved Guideline (1986).** Procedures for evaluating the performance of materials used in immunoprecipitin analysis.
- DI3-A** **Agglutination Analysis: Antibody Characteristics, Methodology, Limitations, and Clinical Validation; Approved Guideline (1993).** Describes specificities of antibodies and their required potency, labeling information, and characteristics and limitations of agglutination methods.
- DI4-T** **Enzyme and Fluorescence Immunoassays; Tentative Guideline (1986).** Methods of achieving reliable, reproducible results from enzyme and fluorescence immunoassays of macromolecular analytes. Factors that contribute to reliable and reproducible results are emphasized in sections that describe how to choose enzyme fluorescence systems, how to produce, process, purify, and characterize antibodies and antigens, as well as reagent separation techniques, instrumentation, and equipment.
- I/LA18-A** **Specifications for Immunological Testing for Infectious Diseases; Approved Guideline (1994).** Guideline that outlines specimen requirements, performance criteria, algorithms for the potential use of sequential or duplicate testing, recommendations for intermethod comparisons of immunological test kits for detecting infectious diseases, and specifications for development of reference materials.
- RS13-P** **Rubella Antibody; Proposed Summary of Methods and Materials Credentialed by the NRSC Council (1989).**

[†] Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.