# Development of Human Anti-Rubella IgG and IgM Assays for the Abbott ARCHITECT® Instrument*

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## Abstract

### Objectives

Develop a panel of human anti-Rubella immunoassays on a high throughput automated platform. The ARCHITECT Rubella IgG assay is intended to be used for the quantitative measurement of IgG antibodies to Rubella virus in human sera or plasma to aid in the determination of immune status to Rubella virus. The ARCHITECT Rubella IgM assay is intended to be used for the qualitative measurement of IgM antibodies to Rubella virus in human serum or plasma to aid in the diagnosis of primary or acute Rubella infection.

### Methods

The prototype Rubella assays for the ARCHITECT instrument are two-step immunoassays utilizing Rubella whole virus-coated paramagnetic microparticles for the capture of anti-Rubella antibodies. An acridinium-labeled monoclonal antibody conjugate directed against human IgG or IgM is utilized for detection. Samples from pregnant women, blood donors, hospital patients, prescreened Rubella IgG negative samples, vaccine serial bleeds, and seroconversion panels have been tested on the new ARCHITECT Rubella assays in comparison to the Abbott AxSYM Rubella IgG and IgM assays. Discrepant samples were tested on one additional Rubella IgG or IgM on-market assay and resolved with the consensus.

### Results

The ARCHITECT Rubella IgG shows a resolved relative sensitivity of 99.66% and a resolved relative specificity of 100% compared to AxSYM Rubella IgG on the population described above (n = 1,149). The ARCHITECT Rubella IgM assay shows a resolved relative specificity of 99.83% on a population consisting of pregnant women, blood donors and hospitalized patients (n = 1,803) and a lower reactive rate than AxSYM Rubella IgM.

### Conclusion

The performance of the new ARCHITECT Rubella IgG and IgM assays was comparable to the AxSYM Rubella IgG and IgM assays.

## Introduction

Primary Rubella virus infection is typically a mild self-limiting disease characterized by a maculopapular rash, fever, malaise and lymphadenopathy. Primary Rubella infection acquired during gestation can seriously complicate pregnancy. Intrauterine transmission of primary Rubella infections may severely damage the fetus, especially during the first trimester. The congenitally infected infant may exhibit one or more of a variety of defects collectively known as Congenital Rubella Syndrome (CRS). Among these are low birth weight, cataracts, deafness, congenital heart disease and mental retardation.

The objective of this study was to evaluate a complete fully-automated Rubella serology panel (Rubella IgG and IgM) that was developed on the Abbott ARCHITECT instrument.

## Methods

### Rubella IgG

The assay utilizes Rubella virus coated onto paramagnetic microparticles which are incubated with the patient sample. Following a washing step of the microparticles, the solid phase immunocomplex is then incubated with an acridinium-labeled mouse anti-human IgG monoclonal antibody. The microparticles are then washed and the signal is generated via chemiluminescence. The assay reportable range is 0 – 5,000 IU/mL. The predicate assay used for comparison is the Abbott AxSYM Rubella IgG assay. Samples with discordant results were resolved using the Vidas Rubella IgG assay.

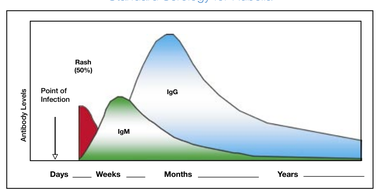
* In development

## Methods

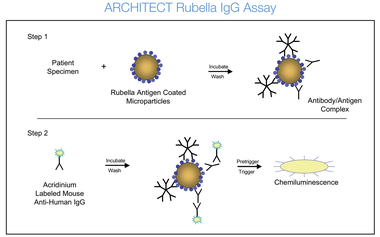
### Rubella IgM

The assay utilizes Rubella virus coated onto paramagnetic microparticles which are incubated with the pretreated patient sample. Following a washing step of the microparticles, the solid phase immunocomplex is then incubated with an acridinium-labeled mouse anti-human IgM monoclonal antibody. The microparticles are then washed and the signal is generated via chemiluminescence. The predicate assay used for comparison is the Abbott AxSYM Rubella IgM assay. Samples with discordant results were resolved using the Vidas and Behring Rubella IgM assays.

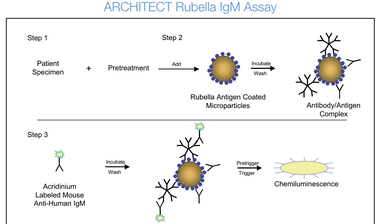
Standard Serology for Rubella




ARCHITECT Rubella IgG Assay




ARCHITECT Rubella IgM Assay

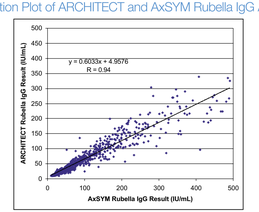



## Results

ARCHITECT Rubella IgG and IgM Imprecision

| Control ID | N | Mean | Within-Run | | Between-Run | | Total | |
|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV |
| ARCHITECT Rubella IgM Negative Control Index | 450 | 0.04 | 0.004 | 9.92 | 0.005 | 10.96 | 0.007 | 15.57 |
| ARCHITECT Rubella IgM Positive Control Index | 450 | 3.75 | 0.069 | 1.85 | 0.108 | 2.88 | 0.143 | 3.82 |
| ARCHITECT Rubella IgG Low Positive Control (25 IU/mL) | 320 | 24.72 | 0.960 | 3.89 | 1.000 | 4.06 | 1.540 | 6.25 |
| ARCHITECT Rubella IgG High Positive Control (300 IU/mL) | 320 | 284.31 | 10.610 | 3.73 | 15.770 | 5.55 | 31.950 | 11.24 |

Correlation Plot of ARCHITECT and AxSYM Rubella IgG Assays




ARCHITECT Rubella IgG Relative Specificity and Sensitivity

| Test population includes prescreened Rubella IgG negative samples, random blood donors, hospital patients and pregnant women (n = 1,149) | | AxSYM Rubella IgG | | |
|---|---|---|---|---|
| | | Positive | Equivocal | Negative |
| ARCHITECT Rubella IgG | Positive | 870 | 0 | 1 |
| | Equivocal | 58 | 39 | 1 |
| | Negative | 7 | 38 | 135 |

Relative Agreement: 99.21% (1005/1013)**
Relative Sensitivity: 99.20% (870/877)**
Relative Specificity: 99.26% (135/136)**
** Equivocal results excluded from calculation

ARCHITECT Rubella IgG Resolved Relative Specificity and Sensitivity

| | | Consensus After Resolution | | |
|---|---|---|---|---|
| | | Positive | Equivocal | Negative |
| ARCHITECT Rubella IgG | Positive | 871 | 0 | 0 |
| | Equivocal | 58 | 39 | 1 |
| | Negative | 3 | 38 | 139 |

Resolved Relative Sensitivity: 99.66% (871/874)**
Resolved Relative Specificity: 100.0% (139/139)**
** Equivocal results excluded from calculation

ARCHITECT Rubella IgM Relative Specificity

| Test population includes random blood donors, hospital patients and pregnant women (n = 1,803) | | AxSYM Rubella IgM | | |
|---|---|---|---|---|
| | | Positive | Equivocal | Negative |
| ARCHITECT Rubella IgM | Positive | 2 | 1 | 3 |
| | Equivocal | 2 | 0 | 0 |
| | Negative | 8 | 0 | 1787 |

Relative Specificity: 99.83% (1787/1790)**
** Equivocal results excluded from calculation

ARCHITECT Rubella IgM Resolved Relative Specificity

| | | Consensus After Resolution | | |
|---|---|---|---|---|
| | | Positive | Equivocal | Negative |
| ARCHITECT Rubella IgM | Positive | 2 | 1 | 3 |
| | Equivocal | 2 | 0 | 0 |
| | Negative | 0 | 0 | 1795 |

Relative Specificity: 99.83% (1795/1798)**
** Equivocal results excluded from calculation

Seroconversion Sensitivity Comparison of the ARCHITECT and AxSYM Rubella IgG, IgM Assays

| | | | | ARCHITECT | | AxSYM | |
|---|---|---|---|---|---|---|---|
| | | | | Rubella IgM (S/CO) | Rubella IgG (IU/mL) | Rubella IgM (S/CO) | Rubella IgG (IU/mL) |
| | | | Cutoff | 1.00 | 10.0 | 1.00 | 10.0 |
| | | | Grayzone to | 0.75 | 5.0 | 0.75 | 5.0 |
| Panel | Bleed | Day | | | | | |
| RPD01 | 1 | 0 | | 0.06 | 0.04 | 0.29 | 3.50 |
| | 2 | 2 | | 0.07 | 0.07 | 0.39 | 1.50 |
| | 3 | 7 | | 0.06 | 0.03 | 0.30 | 1.40 |
| | 4 | 9 | | 0.05 | 0.00 | 0.18 | 0.50 |
| | 5 | 14 | | 0.12 | 0.23 | 0.29 | 0.70 |
| | 6 | 17 | | 0.81 | 3.23 | 0.60 | 1.40 |
| | 7 | 21 | | 5.41 | 20.55 | 3.43 | 12.10 |
| | 8 | 24 | | 4.24 | 27.76 | 3.26 | 24.80 |
| | 9 | 28 | | 2.98 | 38.98 | 2.11 | 47.40 |
| | 10 | 31 | | 2.52 | 71.51 | 2.40 | 97.10 |
| | 11 | 35 | | 2.04 | 61.00 | 1.90 | 89.60 |
| | 12 | 38 | | 1.79 | 50.58 | 1.21 | 60.80 |
| | 13 | 42 | | 1.29 | 48.98 | 1.17 | 56.20 |
| | 14 | 45 | | 1.19 | 44.61 | 0.87 | 48.60 |
| | 15 | 50 | | 0.88 | 42.52 | 0.75 | 54.70 |

| | | | | ARCHITECT | | AxSYM | |
|---|---|---|---|---|---|---|---|
| | | | | Rubella IgM (S/CO) | Rubella IgG (IU/mL) | Rubella IgM (S/CO) | Rubella IgG (IU/mL) |
| | | | Cutoff | 1.00 | 10.0 | 1.00 | 10.0 |
| | | | Grayzone to | 0.75 | 5.0 | 0.75 | 5.0 |
| Panel | Bleed | Day | | | | | |
| RPD11 | 1 | 0 | | 0.03 | 0.04 | 0.24 | 0.60 |
| | 2 | 3 | | 0.03 | 0.04 | 0.20 | 0.30 |
| | 3 | 9 | | 0.03 | 0.09 | 0.16 | 0.40 |
| | 4 | 12 | | 0.04 | 0.03 | 0.17 | 0.60 |
| | 5 | 16 | | 0.92 | 3.19 | 0.92 | 4.60 |
| | 6 | 19 | | 3.27 | 30.44 | 3.44 | 35.20 |
| | 7 | 24 | | 2.96 | 86.87 | 5.10 | 112.50 |
| | 8 | 27 | | 2.37 | 97.66 | 4.25 | 145.80 |
| | 9 | 31 | | 1.88 | 78.68 | 2.78 | 120.40 |
| | 10 | 36 | | 1.41 | 85.42 | 1.75 | 158.20 |
| | 11 | 39 | | 0.98 | 86.97 | 1.42 | 164.20 |
| | 12 | 43 | | 0.75 | 83.81 | 0.97 | 164.30 |
| | 13 | 46 | | 0.61 | 88.45 | 0.73 | 134.50 |
| | 14 | 50 | | 0.49 | 83.70 | 0.63 | 138.00 |
| | 15 | 53 | | 0.44 | 83.90 | 0.53 | 139.40 |
| | 16 | 57 | | 0.34 | 84.46 | 0.58 | 171.90 |
| | 17 | 60 | | 0.27 | 76.56 | 0.33 | 147.50 |
| | 18 | 64 | | 0.22 | 74.37 | 0.28 | 159.20 |
| | 19 | 67 | | 0.20 | 72.51 | 0.28 | 157.20 |
| | 20 | 71 | | 0.16 | 82.11 | 0.22 | 163.10 |

| | | | ARCHITECT | | AxSYM | |
|---|---|---|---|---|---|---|
| | | | Rubella IgM (S/CO) | Rubella IgG (IU/mL) | Rubella IgM (S/CO) | Rubella IgG (IU/mL) |
| | | Cutoff | 1.00 | 10.0 | 1.00 | 10.0 |
| | | Grayzone to | 0.75 | 5.0 | 0.75 | 5.0 |
| Panel | Bleed | Day | | | | |
| RPD14 | 1 | 0 | 0.04 | 0.00 | 0.21 | 0.90 |
| | 2 | 5 | 0.03 | 0.03 | 0.22 | 1.00 |
| | 3 | 7 | 0.03 | 0.07 | 0.17 | 0.10 |
| | 4 | 12 | 0.06 | 0.17 | 0.29 | 1.30 |
| | 5 | 14 | 0.24 | 1.47 | 0.27 | 1.60 |
| | 6 | 19 | 1.57 | 40.36 | 1.24 | 30.50 |
| | 7 | 21 | 1.84 | 52.27 | 1.41 | 52.50 |
| | 8 | 26 | 1.66 | 52.85 | 1.21 | 82.20 |
| | 9 | 28 | 1.42 | 72.54 | 1.45 | 90.20 |
| | 10 | 33 | 0.91 | 97.55 | 1.23 | 75.70 |
| | 11 | 35 | 0.91 | 74.09 | 0.87 | 87.40 |
| | 12 | 40 | 0.66 | 58.61 | 0.55 | 97.50 |
| | 13 | 42 | 0.58 | 85.42 | 0.65 | 115.90 |

Serial Bleed Sensitivity Comparison of the ARCHITECT and AxSYM Rubella IgG, IgM Assays

| | | | | ARCHITECT | | AxSYM | |
|---|---|---|---|---|---|---|---|
| | | | | Rubella IgM (S/CO) | Rubella IgG (IU/mL) | Rubella IgM (S/CO) | Rubella IgG (IU/mL) |
| | | | Cutoff | 1.00 | 10.0 | 1.00 | 10.0 |
| | | | Grayzone to | 0.75 | 5.0 | 0.75 | 5.0 |
| Histogram Number | Sample ID | Date of Specimen Collection | Gestational Age (First Day of Last Menstrual Period, LMP) | | | | |
| | | | (3/17/1998) | | | | |
| 1490 | 98 05 0430 | 5/8/1998 | 7 weeks 3 days | 2.94 | 222.10 | 2.90 | 219.00 |
| | 98 06 0290 | 6/4/1998 | 11 weeks 2 days | 3.34 | 213.90 | 2.53 | 254.00 |
| | 98 07 0252 | 7/3/1998 | 15 weeks 3 days | 3.04 | 210.70 | 2.39 | 305.00 |
| | | | (12/28/1998) | | | | |
| 1618 | 99 01 1449 | 1/29/1999 | 4 weeks 4 days | 1.19 | 49.90 | 0.34 | 61.40 |
| | 99 02 0999 | 2/22/1999 | 8 weeks 0 days | 1.14 | 52.90 | 0.39 | 54.70 |
| | Vaccination | 5/21/1999 | (4/15/1999) | | | | |
| 1643 | 99 06 0385 | 6/8/1999 | 7 weeks 5 days | 4.15 | 5.40 | 2.93 | 6.50 |
| | 99 07 0141 | 7/2/1999 | 11 weeks 1 day | 3.84 | 68.40 | 2.63 | 93.90 |
| | | | (4/30/1999) | | | | |
| 1653 | 99 06 1014 | 6/22/1999 | 7 weeks 4 days | 2.27 | 204.30 | 1.08 | 500.00 |
| | 99 08 0814 | 8/19/1999 | 15 weeks 6 days | 2.09 | 177.90 | 1.09 | 380.00 |

Before and After Vaccination Sensitivity Comparison of the ARCHITECT and AxSYM Rubella IgG, IgM Assays

| | | | | ARCHITECT | | AxSYM | |
|---|---|---|---|---|---|---|---|
| | | | | Rubella IgM (S/CO) | Rubella IgG (IU/mL) | Rubella IgM (S/CO) | Rubella IgG (IU/mL) |
| | | | Cutoff | 1.00 | 10.0 | 1.00 | 10.0 |
| | | | Grayzone to | 0.75 | 5.0 | 0.75 | 5.0 |
| Sample ID | Date of Vaccination | Date of Specimen | Weeks After Vaccination | | | | |
| 98 03 0542 | 4/6/1998 | 3/11/1998 | | 0.03 | 0.30 | 0.12 | 0.00 |
| 99 02 1120 | | 2/24/1999 | 46 weeks 2 days | 0.10 | 63.60 | 0.35 | 88.20 |
| 98 01 0665 | 1/25/1999 | 1/13/1998 | | 0.03 | 4.50 | 0.12 | 4.00 |
| 99 03 0195 | | 3/2/1999 | 5 weeks 1 day | 0.03 | 18.20 | 0.16 | 18.40 |
| 98 11 0225 | | 11/4/1998 | | 0.11 | 0.10 | 0.15 | 0.20 |
| 98 12 0696 | 11/16/1998 | 12/14/1998 | 4 weeks 0 days | 10.32 | 47.50 | 8.03 | 56.70 |
| 99 03 1448 | | 3/26/1999 | 18 weeks 4 days | 2.41 | 81.60 | 1.83 | 97.20 |
| 98 09 0684 | 3/15/1999 | 9/11/1998 | | 0.04 | 3.60 | 0.12 | 5.30 |
| 99 05 0423 | | 5/10/1999 | 8 weeks 0 days | 0.10 | 18.50 | 0.18 | 41.20 |
| 99 01 1127 | 3/16/1999 | 1/22/1999 | | 0.03 | 0.10 | 0.11 | 0.00 |
| 99 05 0905 | | 5/20/1999 | 9 weeks 2 days | 0.48 | 89.80 | 0.84 | 127.00 |
| 99 06 0360 | 6/25/1999 | 6/8/1999 | | 0.04 | 0.20 | 0.16 | 0.40 |
| 99 07 1070 | | 7/27/1999 | 4 weeks 4 days | 0.68 | 7.00 | 1.17 | 6.70 |
| 99 11 0977 | 12/16/1999 | 11/18/1999 | | 0.04 | 0.00 | 0.10 | 0.00 |
| 20 01 1482 | | 1/28/2000 | 6 weeks 1 day | 1.33 | 17.20 | 1.74 | 24.80 |

## Conclusions

- The performance of the ARCHITECT Rubella IgG and IgM assays was comparable to the AxSYM Rubella IgG and IgM assays.
- The reactive rate of the ARCHITECT Rubella IgM assay was less than the AxSYM Rubella IgM assay.
- The Congenital panel on the ARCHITECT instrument, in addition to the Rubella IgG and IgM assays, will also include the CMV and Toxo IgG, IgM and IgG avidity assays.