

AN ARCHITECT ASSAY FOR MEASUREMENT OF CYFRA21-1

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Abstract

CYFRA 21-1, the soluble fragments of cytokeratin 19, has been shown to be a useful biomarker for lung cancer. An ARCHITECT CYFRA 21-1 assay is under development and the analytical performance of the assay is described.

Methods: Samples (serum or plasma) and antibody KS19.1-coated paramagnetic microparticles were combined and incubated first. Following a wash, acridinium-labeled antibody BM19.21 conjugates were added to the mixture. The chemiluminescence relative light units, which were generated after adding Pre-trigger and Trigger solutions into the reaction mixture, were used to determine the amount of CYFRA 21-1 antigen in the samples.

Results: The assay has a calibration range of 0-100 ng/mL. With CYFRA 21-1 values obtained from ELECSYS System (Roche) as the reference, Passing & Bablok fit deduced a correlation as [ARCHITECT] = 1.00*[ELECSYS] - 0.45 using serum samples (N = 200) within the calibration range. Interference studies showed the differences between the measured concentrations and the expected values (target value 3.3 ng/mL) of CYFRA 21-1 were less than or equal to 10% in RF or HAMA-positive sera, sera (N = 5) with individually added endogenous interference materials (12 g/dL protein, 20 mg/dL bilirubin, 3,000 mg/dL lipid and 5 mg/mL hemoglobin) and pooled sera individually added with each of 14 therapeutic drugs. Buffer-based controls (5, 15 and 35 ng/mL) and serum or plasma-based panels (1.5, 2.1, 2.9, 15.5 and 66.8 ng/mL) were developed and imprecision studies are to be carried out using the controls and panels.

Conclusion: The ARCHITECT CYFRA 21-1 assay appears to be a promising automated assay for the measurement of circulating CYFRA 21-1.

Introduction

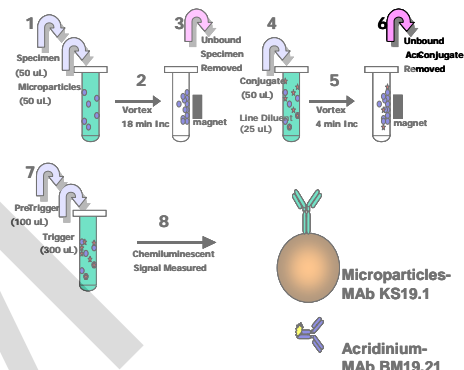
CYFRA 21-1

- One of the most sensitive tumor markers in serum from patients with non-small cell lung cancer (NSCLC).
- Research reports showed its usefulness in differential diagnosis, prognosis, therapy monitoring and detection of recurrent disease for NSCLC.
- ARCHITECT CYFRA 21-1 is a quantitative assay measuring soluble fragments of cytokeratin 19 (CYFRA 21-1) using monoclonal antibodies, BM19.21 and KS19.1.

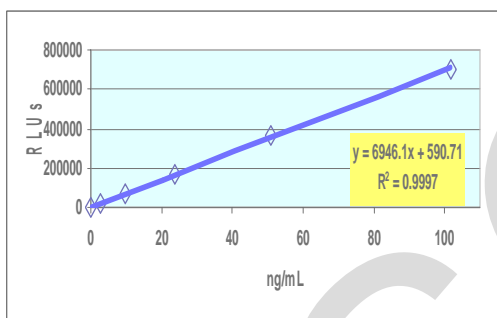
References: Bodenmuller H. 1994, Stieber P. 1998; Nilsson O. 2008

ARCHITECT ASSAY

- A leading automated format for immunologic assay with excellent sensitivity, precision and accuracy.

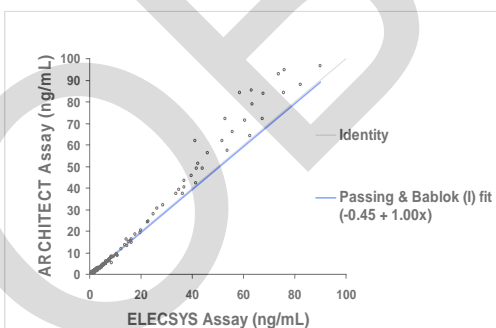


Calibration Curve



Calibration Range: 0 – 100 ng/mL

Method Comparison



ARCHITECT CYFRA 21-1 assay is in good agreement to ELECSYS CYFRA 21-1.

Endogenous Interference

- Sera with known values of rheumatoid factor or HAMA were spiked with CYFRA 21-1 targeting 3.3 ng/mL.
- Additionally sera from normal individuals were spiked with CYFRA 21-1 and targeted 3.3 ng/mL. Then Bilirubin, protein, intralipid or hemoglobin were individually spiked into the sera.
- Differences between the measured vs. assigned values of CYFRA 21-1 are shown.

Endogenous Materials	Sera (N)	% Differences for CYFRA 21-1
26 - 279 U/mL RF	10	≤ 9%
40 -122 U/mL HAMA	10	≤ 9%
12 g/dL Protein	5	≤ 6%
3000 mg/dL Lipid	5	≤ 10%
5 mg/mL Hemoglobin	5	≤ 10%
20 mg/dL Bilirubin	5	≤ 7%

6 endogenous interfering factors demonstrated < 10% differences.

Therapeutic Interference

Therapeutics	ug/mL	CYFRA 21-1		
		Control (ng/mL)	With Therapeutics (ng/mL)	Difference (%)
Paclitaxel	67	3.9	4.0	1%
Docetaxel	1	3.9	3.9	0%
Gemcitabine	380	4.1	4.3	4%
Vinorelbine	1.23	3.8	3.9	2%
Irinotecan	20	4.1	4.2	2%
Etoposide	12	3.9	4.0	2%
Vinblastine	500	3.9	4.1	4%
Erlotinib	17	4.3	4.2	2%
Pemetrexed	1370	3.8	3.9	4%
Cisplatin	165	3.1	3.2	5%
Carboplatin	500	4.1	4.2	1%
Doxorubicin	40	4.0	4.1	3%
Vincristine	0.011	4.1	4.1	0%
Ifosfamide	800	4.2	4.2	0%

14 therapeutics commonly used to treat lung cancer demonstrated a difference ≤ 5%.

Conclusions

The performance of the ARCHITECT CYFRA 21-1 Assay in development is:

- in good agreement to ELECSYS CYFRA 21-1 Assay: Slope = 1.00;
- minimally influenced by therapeutics and endogenous materials;

References

- Bodenmuller H. et al. Int J Biol Markers 1994; 9: 75-81.
- Stieber P. in Thomas L., ed. Clinical Laboratory Diagnostics 1998, 966-970.
- Nilsson O. et al. Tumor Biology 2008; Abstracts of the 36th Meeting of ISOBM P2-07.

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