Real-time Analysis of RAM Amplification
Using RAM-Specific Strategies, and Methodologies Developed for PCR
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Abstract
PCR amplification efficiency and RAM amplification rate are two measures that must be accounted for to make accurate sample-to-sample comparisons in real-time amplification systems. PCR and RAM real-time data can be analyzed with mathematical models that provide estimates of PCR efficiency or RAM reaction rate. RAM reactions can be sampled at high density, yielding sufficient data for direct fitting of a simple exponential model.

RAM, like PCR, is an amplification method.

RAM amplification (Zhang et al., 1999) and RAM product detection are the final steps in a molecular test (DNA or RNA detection process. Table 1 compares features of PCR and RAM. Figure 1 illustrates mechanisms of amplification in the two systems. Both systems use two primers in a two-primer continuous process of primer extension and displacement. Briefly: theoretical, the RAM reaction is an isothermal reaction.

A substantial literature (Pfaffl, 2001; Peirson et al., 2003) and reference collection at "A base-curve (5-parameter log-logistic; Spiess et al., 2008), shown as a solid line, is a model curve for data of exponential amplification. The solid line is the data that was used to generate the model curve by manual least-squares fit. AMR reaction fluorescence data from three amplification reactions. The data collection frequency yields sufficient temporal resolution for all data points to be visible in the display. The time defined by the intersection of the extrapolated baseline and extrapolated exponential phase derived from logistic fit parameters; 2, second derivative crossing point; 1, first derivative crossing point.

Figure 5A shows amplifications from well-separated dilutions chosen from Figure 4A, with the same data set as in Figure 4A. Three amplifications show an exponential curve, while one shows an exponential curve followed by a phase that is linear to the baseline. The response-time is marked by an arrow (Rt) indicating the intersection of the extrapolated baseline and extrapolated exponential phase derived from logistic fit parameters. The time defined by the intersection of the extrapolated baseline and extrapolated exponential phase derived from logistic fit parameters; 2, second derivative crossing point; 1, first derivative crossing point.