Application of a real time PCR method to detect castor toxin contamination in fluid milk and eggs

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Abbreviations: Ct, threshold cycle; CTAB, cetyl trimethylammonium bromide; ELISA, enzyme-linked immunosorbent assay; LD_{50}, median lethal dose; R^2, square of regression coefficient; RCA, *Ricinus communis* agglutinin; rRNA, ribosomal RNA; SD, standard deviation.

Abstract

The castor seed contains ricin, which is one of the most potent biological toxins and is widely considered to be a threat agent for bioterrorism. In this study, a rapid and sensitive PCR method was applied to the detection of castor contamination in milk and liquid egg samples. The targeting gene sequence of primer set, ricin-F4/R4 was not found in either the bovine or chicken genome. Primers against a highly conserved sequence from the 18S ribosomal RNA gene were used as a positive control for DNA extraction and PCR reaction efficiency. The quantity and quality of DNA prepared from castor spiked or non-spiked milk and egg samples obtained from three different DNA extraction methods were compared. The cetyl trimethylammonium bromide (CTAB) method yielded the highest quality of DNA and is most suitable for the sensitive detection of castor DNA by real-time PCR in both milk and liquid egg matrices. However, taking time and cost into consideration, a commercial kit designed for extraction of DNA from stool samples could be used as an alternative method for the routine extraction of DNA from milk for real-time PCR assays. The egg matrix was found to inhibit PCR amplification and interfere with two of the three methods tested for DNA extraction. Egg yolk had a greater negative effect on PCR amplification than the egg white matrix. Our results affirm the necessity of performing individual validations for each food matrix. Both real-time PCR systems used in this study, TaqMan and SYBR Green I dye, were capable of detecting 100 ng of castor acetone powder, corresponding to 5 ng of ricin, in 1 mL milk or liquid egg, well below the toxic dose for humans. Based on these results, the real-time PCR method for detection of intentional castor contamination is applicable to milk and egg matrices.

Introduction

The castor plant (*Ricinus communis)* is cultivated mainly for the unique oil contained in the seeds. The oil consists of 90% ricinoleate, an hydroxy fatty acid, and has numerous industrial uses. However, the seed of the castor plant also contains the toxin ricin, one of the most poisonous naturally occurring substances. After oil extraction, the residual castor meal contains about 5% toxin. Although the ricin can be readily purified by a simple affinity chromatographic procedure (*1*), it is generally thought that crude, solvent extracted powder is the likely product to be used, as it requires little technical expertise and very inexpensive household supplies. Ricin, a potent cytotoxin and a weak hemagglutinin, is stored in the endosperm of the bean, together with the weak cytotoxin and powerful hemagglutinin, *R. communis* agglutinin (RCA, often designated RCA_{120} or RCA-1) (*2*). Although the DNA sequences of ricin and RCA are very similar, the transcripts of these two genes are distinguished easily by a reverse transcription-polymerase chain reaction assay (*3*). Ricin is synthesized as a single polypeptide chain that is cleaved to yield the A- and B-chain linked by a single disulfide bond. The ricin A chain is an *N*-glycosidase that binds and depurinates the 28S ribosomal RNA (rRNA) at residue A4324 (*4*), altering the dynamic flexibility of the ribosome and irreversibly disrupting protein synthesis. The B chain is a lectin that binds to galactosyl proteins and lipids on cell membranes,enabling entry of the entire ricin molecule into cells via receptor-mediated endocytosis. The ricin dimer can bind to galactose but is unable to depurinate 28S rRNA (*5*).

The toxicity of ricin varies with the route of exposure. In laboratory mice the median lethal dose (LD_{50}) is 3-5 µg/kg by inhalation and intravenous injection, 22 µg/kg by intraperitoneal injection, and 20 mg/kg by intragastric administration (*6*). There are no literature reports of poisoning from ingesting purified ricin in humans. The lethal oral dose estimated from castor bean ingestion ranged from 2 to 80 mg per adult human (*7, 8*). Because of its high toxicity and facile preparation from castor bean, ricin is a potential threat agent. The possibility that bioterrorism could affect the US food supply using biological toxins has become a concern, and the development of a rapid and sensitive detection method for contamination in foods is therefore urgently needed.

Routine detection of ricin in food is usually carried out by immunological methods including: enzyme-linked immunosorbent assay (ELISA) (*9*), immunochromatographic devices (*10*), and chip-based methods (*11*). In these methods the sensitivity and specificity of detection depends on the stability of the protein and the antibody. Recently, real-time PCR has been applied for the quantitative detection of pathogens (*12, 13*), toxins (*14*) and genetically modified food (*15, 16*) and has proven to be rapid, robust, sensitive and inexpensive.

Most documented ricin poisonings have involved ingestion of castor seeds, and the potential tampering with food by bioterrorists would likely involve crude materials (*7*). In these materials, castor-specific nucleic acids provide appropriate surrogate analytes for ricin. Using real-time PCR, our previous work showed that the limit of detection for crude castor contamination in ground beef was less than 0.001% by weight (*17*), well below the threshold for oral toxicity. In this study, we sought to expand the real-time PCR protocol for detection of crude ricin in liquid foods. These foods contain substances that could interfere with the assay protocol, namely high calcium content (milk) or high emulsifier content (egg). The specific objectives of this study were to: 1) test the specificity of the primers in egg and milk matrices; 2) evaluate the effect of these food matrices on different DNA extraction protocols; 3) determine the limit of detection for ricin DNA sequences in milk and egg matrices using SYBR Green and TaqMan chemistries. On the basis of results obtained, we also examined the inhibitory effect of different egg fractions on PCR amplification.

Materials and Methods

Acetone Powder.

Stable acetone powder from castor seeds was prepared as described by Tewfik and Stumpf (*18*). The freshly prepared powder was dried overnight in a vacuum desiccator over P_2O_5, and stored at –20°C.

DNA extraction.

Milk and eggs were purchased and used within the prescribed shelf-life from a local supermarket and stored at 4°C. Before DNA extraction, whole eggs were homogenized by vortexing. Samples were divided into 1 mL aliquots. Genomic DNA from milk and liquid egg was extracted by 3 different DNA purification methods:

1. CTAB method. The extraction was performed as described by Nemeth et al. (*19*) with slight modification. Briefly, 1 mL milk or egg samples were mixed with an equal volume of CTAB extraction buffer [1.4 M NaCl, 2% CTAB (cetyl trimethylammonium bromide), 100 mM Tris, 20 mM EDTA, pH 8.0, 1% polyvinylpyrrolidone-40], 2% of 2-mercaptoethanol and 100 µg/mL of proteinase K for 1h at 65°C with shaking. The samples were extracted with 2 mL of chloroform, centrifuged for 15 min at 12,000 *g*, and precipitated with 2 volumes of CTAB precipitation buffer (40 mM NaCl, 0.5% CTAB) at room temperature for 60 min and centrifuged for 20 min at 15,000 *g*. The pellet was dissolved in 350 µL of 1.2 M NaCl, and extracted with an equal volume of chloroform. DNA in the aqueous phase was precipitated with 350 µL 2-propanol plus 1 µL glycogen (5 mg/mL). The pellet was washed with 75% ethanol and resuspended in 100 µL water.

2. DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). A sample of 1 mL was mixed with 1 mL ATL buffer and 120 µL of proteinase K. Extraction was performed according to manufacturer's instructions for purification of DNA from animal tissues. DNA was eluted in 100 µL of water.

3. QIAamp DNA Stool Mini Kit (Qiagen). Extraction was performed according to manufacturer's instruction except that the starting material was scaled up to 1 mL and the amount of buffers and other reagents were also increased proportionally. DNA was eluted in 100 µL water.

Oligonucleotides.

Oligonucleotide primer pairs, Ricin-F4, Ricin-R4 for the ricin gene and 18S-F, 18S-R for the 18S rRNA gene were as described previously (*17*). The sequence for the probe to be used with the primer set, Ricin-F4/R4, in the TaqMan PCR assay is: 5'-ATGCAGATGGTTCAATAC-3' at nucleotide position 1779-1796 of the ricin gene (X52908). The probe was attached to the reporter dye, FAM, at the 5' end and a minor

groove-binder at the 3' end with the non-fluorescent quencher. The oligonucleotides were synthesized by MWG-Biotech (Oaks Parkway, NC, USA).

PCR methodology.

For SYBR-Green I dye chemistry, the PCR reactions were carried out as described previously (*17*) using 5 µL of DNA solution in a final volume of 25 µL. For TaqMan chemistry, amplification mixtures contained 1x TaqMan Universal PCR Master Mix (Applied Biosystems), forward and reverse primers (900 nM each), gene-specific probe (250 nM) and 5 µL of DNA solution in a final volume of 25 µL.

Real-time PCR quantification.

Three replicates were carried out for each PCR assay as well as a nontemplate control for each experiment. The threshold for each amplification plot was selected automatically by the software. The cycle threshold (Ct) is defined as the fractional cycle number at which the fluorescence in the reaction passes the threshold. The Ct value is inversely proportional to the amount of DNA template. The amplification efficiency of a real-time PCR assay is determined using the equation: $E = (10^{-1/slope} - 1) \times 100$ (*20*).

Sequencing.

To confirm the PCR results, PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen), cloned using TOPO TA Cloning Kit for Sequencing (Qiagen) and sequenced using ABI3100 DNA analyzer and ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

Spiking food samples with castor materials.

In two replicate experiments for each type of food, milk and liquid egg, 100 µL of phosphate-buffered saline (PBS) containing varying amounts of castor acetone powder was added to 1 mL of each food matrix. Subsequently, DNA extraction was performed. 5 µL of the resulting DNA solution was used for real-time PCR. Three replicate measurements were carried out for each assay. DNA extracts from unspiked milk or egg samples were included as blanks in each experiment.

Results and Discussion

Selectivity of the ricin gene targeted primer pair, Ricin-F4/R4

In the present study, SYBR-Green I dye based real-time PCR was carried out using the primer pair Ricin-F4/R4 (*17*), derived from the castor ricin gene for amplification of ricin DNA fragment in DNA extracted from the unspiked and spiked milk and egg samples using the CTAB method (Fig. 1). An amplicon of expected size, 69 bp, was amplified from milk and egg samples spiked with 0.1% castor acetone powder and confirmed to be the ricin gene. There were no PCR products observed in the assay when the template was genomic DNA extracted from the unspiked milk and egg samples. To confirm the presence of DNA in the unspiked food samples, PCR assays were performed to amplify the 18S rRNA gene using DNA templates extracted from spiked and unspiked milk and egg samples. PCR products of the expected size were observed in all PCR reactions. The primer pair used, 18S-F/R, was originally derived from the sequence of the *R.communis* 18S rRNA gene. Alignment of DNA sequences from castor, bovine and chicken indicated that the sequence of the region for primer 18S-F is identical among these species. There is a 3-base difference in the primer region of 18S-R between castor and bovine or chicken, but no difference between bovine and chicken. These results indicated that DNA prepared from the different samples was PCR amplifiable and that the primer pair, 18S-F/R, served effectively as universal primers. However, the primer pair Ricin-F4/R4 was castor-specific and therefore is useful for detection of castor contamination.

Because SYBR-Green I dye chemistry detects all double-stranded DNA, including non-specific PCR products and primer-dimers, it is essential to perform dissociation-curve analysis to confirm the melting temperature (T_m) of a single target nucleic acid sequence within an unknown sample. Figure 2 displays the melting curves associated with the dissociation analyses from PCRs. The curves obtained from PCR assays using the primer pair, Ricin-F4/R4, SYBR Green I dye and DNA from milk and egg samples spiked with castor acetone powder were similar to those obtained using castor genomic DNA as template, as previously observed (*17*). A single amplification peak from the specific product had a T_m of 75.7°C, demonstrating that there were no primer-dimers or additional nonspecific products present in these PCR reactions. There was no milk or egg matrix

effect on the specificity of PCR amplification of ricin using Ricin-F4/R4 as primers and DNA extracted by the CTAB method as template.

Evaluation of three different extraction methods for castor DNA in spiked milk and egg samples

The advantages of real-time PCR as a detection method are high sensitivity and speed. However, as with other methodologies, suitable preparation of the samples is the key for PCR to succeed. PCR can be compromised by excessive amounts of PCR inhibitors such as chelators of cations and substances that bind or degrade the polymerase or DNA template. Because foods vary greatly in their composition, the effective isolation of total DNA presents a challenge. For example, milk contains high levels of cations (Ca++), proteases, nucleases, fatty acids and DNA (*21*); while liquid egg contains high levels of protein and lipid, several biologically active albumen proteins such as lysozyme, protease inhibitors and binding proteins (*22*). Unique PCR inhibitors are found in most foods, including milk and egg (*23*). Therefore, DNA extraction protocols must be individually validated for different sample matrices.

Three DNA purification methods (CTAB, DNeasy Tissue Kit and DNA Stool Mini Kit) were evaluated for milk and liquid egg. The extraction procedures were optimized using 1 mL of starting sample spiked with 0.1% castor acetone powder, and DNA product was dissolved or eluted in 100 μL of water. The DNeasy Tissue Kit and DNA Stool Mini Kit provided rapid DNA purification from samples, but the A_{260}/A_{280} was much lower than that of pure DNA (1.8-2.0), suggesting the presence of UV-absorbing contaminants, such as protein. The CTAB extraction method yielded a higher concentration of DNA (22 and 12 ng/μL from milk and egg, respectively), with acceptable DNA purity (A_{260}/A_{280} was 1.8 for both matrices) (Table 1).

The quantity and quality of DNA was also evaluated via the SYBR Green I dye based real-time PCR assay using each of the three extraction protocols. Because the Ct value in real-time PCR is inversely proportional to the amount of target DNA in the initial PCR reaction, the Ct values for the ricin and 18S-rRNA genes can be used as an indication of the amount of genomic DNA recovered from the sample. Figure 3 shows the Ct values obtained for the 18S-rRNA and ricin genes using 5% of the isolated DNA as template in PCR reactions. No ricin was detected in unspiked milk and egg samples (data not

shown). In milk samples spiked with castor acetone powder, the CTAB method gave the best results based on lower Ct values for both ricin and 18S-rRNA genes, as the other two methods gave Ct values 1 to 4 cycles higher (Fig.3). However, isolation of DNA using the DNA Stool Mini Kit was rapid and may be a better choice if the processing time is critical. The DNA Stool method took half the time for DNA extraction, and the increase in Ct value was only 1-2 cycles. For spiked egg samples, the Ct values from CTAB method were 23 for the ricin and 18S rRNA genes, but the Ct values for both ricin and 18S-rRNA genes corresponding to the DNeasy Tissue Kit and DNA Stool Mini Kit were greater than 40, beyond the detection limit of this assay (data not shown). When the amount of template from the DNeasy Tissue Kit was decreased by half, the Ct values for the ricin and 18S rRNA genes became detectable (36 and 34), suggesting the presence of PCR inhibitors in the DNA template. Therefore, the CTAB method provided the best quality template for detection of castor contamination in liquid egg.

PCR inhibition by egg components

Liquid egg consists of two main parts, yolk and white, differing significantly in composition. On a dry weight basis, egg white contains over 80% protein, while the egg yolk consists of two-thirds lipid and one-third protein. To evaluate the inhibitory effect of egg white and yolk on DNA extraction and PCR amplification, 1 mL of the two egg fractions were spiked with 0.0001% of castor acetone powder, and the DNA was extracted using the CTAB method. Table 2 shows the DNA concentration and Ct values for the ricin and 18S rRNA genes obtained by SYBR-Green I-based real-time PCR from different fractions of the egg. Based on A_{260}, egg yolk contains more DNA than egg white, but the Ct value for the 18S rRNA gene was identical for egg yolk and white. The Ct value for the ricin gene in spiked egg yolk was consistently 3 cycles higher than that of egg white. Although the same amount of template was added to egg yolk and white, greater inhibition caused by factors in the egg yolk led to a higher Ct values obtained for egg yolk than for egg white, suggesting that egg yolk contains more PCR inhibitors than egg white. Similar experiments were performed to determine the inhibitory effects of milk fat (0-4%) on DNA extraction and SYBR-Green I-based real-time PCR, but no significant differences were observed (data not shown).

Limit of detection for castor contamination in spiked milk and egg samples using SYBR Green I and TaqMan systems

The limit of detection was determined by adding a 10-fold serial dilution of castor acetone powder to milk and liquid egg samples (ranging from 0.00001 to 1%), followed by DNA extraction using the CTAB method and real-time PCR. Figure 4 shows the average Ct values obtained with SYBR-Green I and TaqMan systems versus the log concentration of castor acetone powder in milk (Fig. 4A) and eggs (Fig. 4B) (μg/mL) for three replicate PCR measurements. On a semi-logarithmic plot, all PCR dose-response curves were linear with $R^2 > 0.99$. The dynamic range of the assay is five orders of magnitude. For milk, the amplification efficiencies estimated from the slope were 95% with SYBR-Green I and 86% with TaqMan, indicating the SYBR-Green I-based real-time PCR assay is more efficient. For liquid egg, the efficiencies were 104% with SYBR-Green I and 100% with TaqMan, suggesting that both assays are optimal or close to optimal for amplification efficiency. However, SYBR-Green I may be more convenient and cost effective for routine detection of castor contamination in liquid egg, because it has sensitivity equivalent to TaqMan, but does not require synthesis of dye-labelled probes. Based on the PCR results from milk samples, the average Ct value for the 18S rRNA gene was approximately 16, and was independent of the amount of castor acetone powder added (from 0.00001 to 1%). However, liquid egg samples yielded Ct values for the 18S rRNA gene that decreased from 27 to 20 as castor acetone powder was increased from 0.00001 to 1%. This may be explained by the relatively abundant DNA found in milk, so that addition of small amounts of castor acetone powder in the sample does not affect PCR results for the 18S rRNA gene. On the other hand, non-fertilized liquid egg contains minor amounts DNA. Therefore, the amount of castor acetone powder added greatly affects the yield of DNA extracted, resulting in the change of Ct values for the 18S rRNA gene.

As little as 0.00001% of castor acetone powder added to 1 mL of milk or liquid egg can be detected using either SYBR-Green I or TaqMan systems. Results obtained with milk showed that the average Ct value for the ricin gene was 38 with SYBR-Green I and 39 with TaqMan at this concentration. The average Ct value in spiked samples of liquid egg

264 was 35 with SYBR-Green I and 38 with TaqMan at the same concentration. The
265 quantification to these low limits in both systems is probably facilitated by the presence
266 of multiple copies of the target sequence (*24*). No detectable reporter amplification was
267 observed using DNA templates from unspiked food samples in the PCR assay,
268 confirming the specificity of the assay for the ricin gene.
269

270 **Conclusions**

271 The purpose of this study was to determine the general applicability of our previously
272 developed method to detect adulteration of foodstuffs with castor seed material that might
273 contain ricin. A real-time PCR assay was applied to detect the nucleic acid that remains
274 associated with these castor materials. The sequence targeted was a fragment of the ricin
275 gene. It has been demonstrated that the primer set, Ricin-F4/R4 derived from the ricin
276 gene, is castor- specific and not found in bovine or chicken. In this assay, a highly
277 conserved sequence from the 18S rRNA gene was included as a positive control, in order
278 to prevent false-negative results that might occur as a consequence of PCR inhibitors.

279 To detect castor DNA in milk and liquid egg, three different DNA extraction methods
280 were evaluated to provide template for amplification of the ricin gene by real-time PCR.
281 Positive results were obtained for milk spiked with 0.1% of castor acetone powder using
282 all three methods. For spiked liquid egg, only the CTAB method gave positive results.
283 These results reaffirm the necessity of validating a given DNA extraction method for
284 each food matrix of concern. In general, of the three methods tested, the CTAB method
285 is the best for isolation of castor genomic DNA directly from milk and liquid egg for real-
286 time PCR detection. However, taking into consideration the time used for extraction, the
287 DNA Stool Mini Kit can be used as a rapid alternative method to extract DNA from
288 potentially contaminated milk.

289 Egg contains components that are highly inhibitory to PCR. Following the prescribed
290 protocols, the DNA extracted by two commercial kits was not amplifiable by PCR. We
291 found the Ct value obtained by SYBR Green I dye-based real-time PCR for the ricin gene
292 was consistently higher in egg yolk than white, although the same amount of castor

material was added to the egg yolk and egg white samples. This result suggests that egg yolk contains substances that interfere with PCR.

The limit of detection for castor contamination in milk and liquid egg was 1 part in 10 million, using both SYBR Green I dye and TaqMan systems in combination with the CTAB extraction method and primer pair, Ricin-F4/R4. This sensitivity is much greater than that for castor contamination in ground beef (*17*). The limit of detection corresponds to 24 µg castor acetone powder (about 1.2 µg of ricin, based on 5% ricin in castor acetone powder, our unpublished data) in a typical one cup serving of milk or 5 µg castor acetone powder (about 0.25 µg of ricin) in one egg. These levels are much lower than the lethal oral dose in human (2-80 mg/per adult) (*7, 8*) . Therefore, the real-time PCR assay we developed here can be a fast and cost effective alternate method for detection of deliberate castor contamination in milk and egg matrices.

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373

374 **Figure legends**

375

376 Figure 1. Real-time PCR was performed using Ricin-F4/R4 (top panel) and 18S-F/R
377 (bottom panel) primers and DNA (5 μL) extracted with the CTAB method from castor,
378 milk or liquid egg as template. The ethidium bromide stained 3% agarose gel with 10 μL
379 of real-time PCR end products is shown. L, 25-bp DNA ladder; 1, nontemplate control;
380 2, DNA from milk; 3, DNA from milk spiked with 0.1% castor bean acetone powder
381 (CAP); 4, DNA from egg; 5, DNA from egg spiked with 0.1% CAP; 6, DNA from castor.

382

383 Figure 2. Dissociation curve analysis confirming that the melting temperature (75.7 ºC)
384 of PCR product obtained from assays using DNA template from milk and liquid egg
385 spiked with 0.1% castor acetone powder were similar to those obtained using castor
386 genomic DNA as template (16).

387

388 Figure 3. Cycle threshold values for the measurements of ricin and 18S rRNA genes by
389 SYBR Green I dye-based real-time PCR using DNA template extracted from milk spiked
390 with 0.1% of castor acetone powder by CTAB method, DNeasy Tissue Kit and DNA
391 Stool Mini Kit. Values are means of three replicates with standard deviation.

392

393 Figure 4. Detection of ricin DNA by PCR. Linear regressions of cycle threshold values
394 on log (μg castor acetone powder) spiked in milk (A) and liquid egg (B). Ct values are
395 means of three replicates with standard deviation.

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400

Table 1. DNA extracted by alternative protocols*

	Milk		Egg	
Extraction protocol	**A_{260}/A_{280}**	**DNA (ng/µL)**	**A_{260}/A_{280}**	**DNA (ng/µL)**
CTAB	1.8 ± 0.11	22 ± 1.05	1.8 ± 0.09	11.8 ± 0.30
DNeasy Tissue Kit	1.4 ± 0.13	5.8 ± 0.25	0.9 ± 0.09	98.0 ± 2.38
DNA Stool Mini Kit	1.5 ± 0.02	4.1 ± 0.28	1.4 ± 0.02	8.0 ± 0.66

*DNA yield calculated from A_{260}. All data are mean ± SD (n=3).

Table 2. Quantity and quality of DNA extracted from different egg fractions[a]

Parameter	Egg white	Egg yolk	Whole egg
DNA (ng/µL)[b]	2.6 ± 0.34	8.7 ± 0.38	5.1 ± 0.42
Ct value for ricin gene	32 ± 0.31	35 ± 0.36	33 ± 0.17
Ct value for 18S rRNA gene	25 ± 0.16	25 ± 0.52	25 ± 0.15

[a] All data are mean ± SD (n=3).

[b] DNA yield calculated from A_{260}.

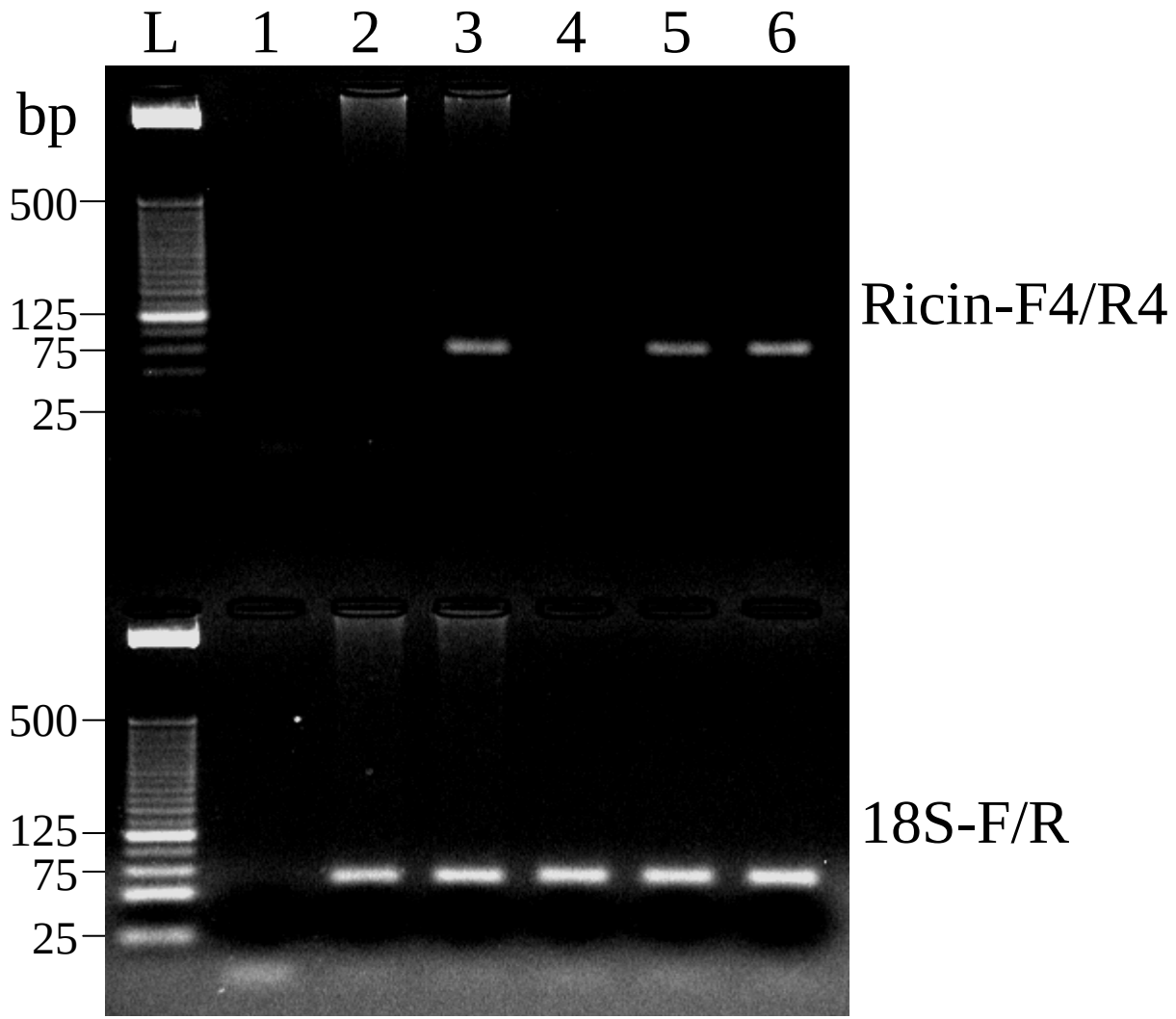
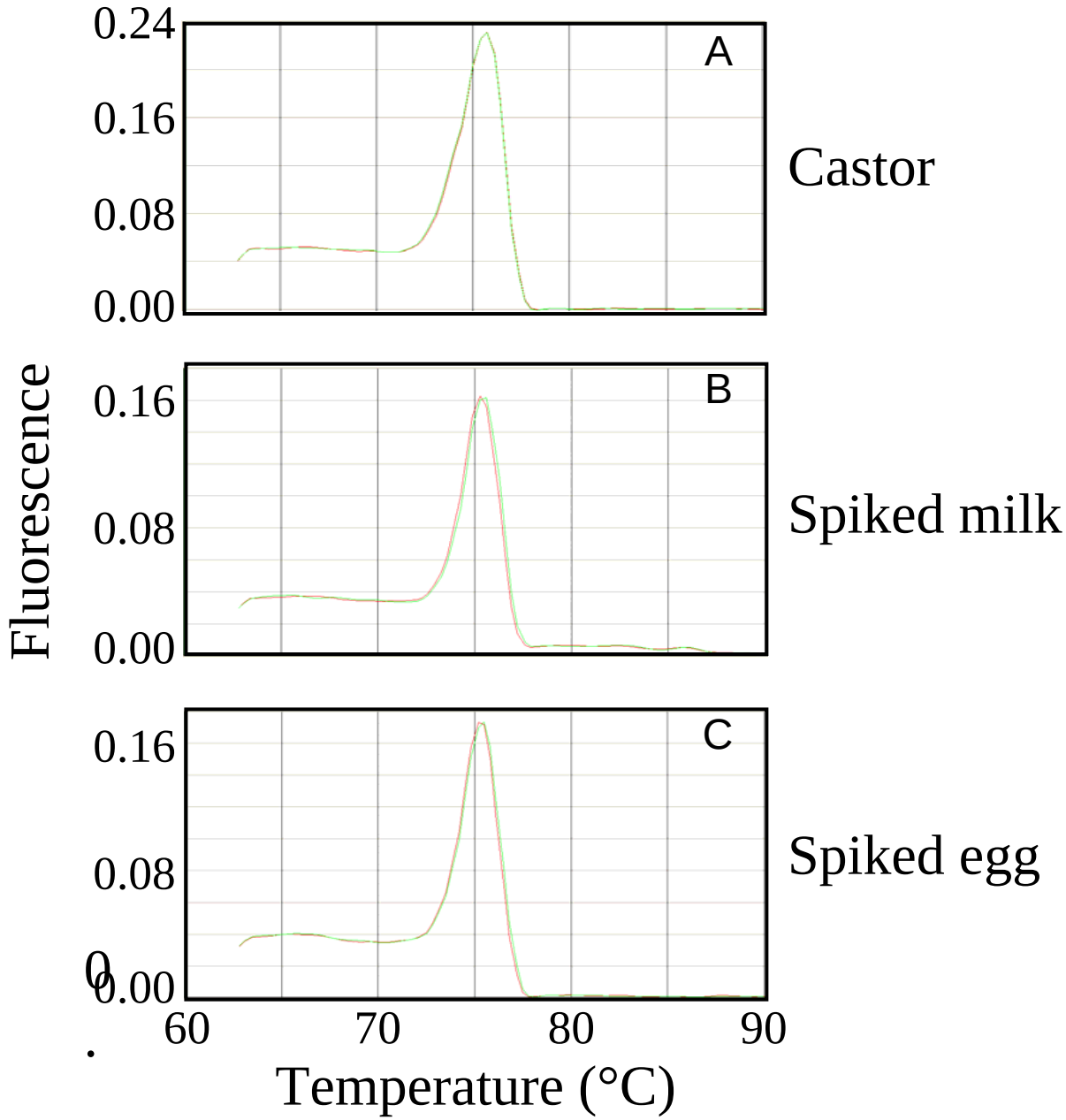


Fig.1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0.00
A
Castor
0.16
0.08
0.00
B
Spiked milk
Fluorescence
0.16
0.08
0.00
C
Spiked egg
0
60
70
80
90
Temperature (°C)

Fig. 2

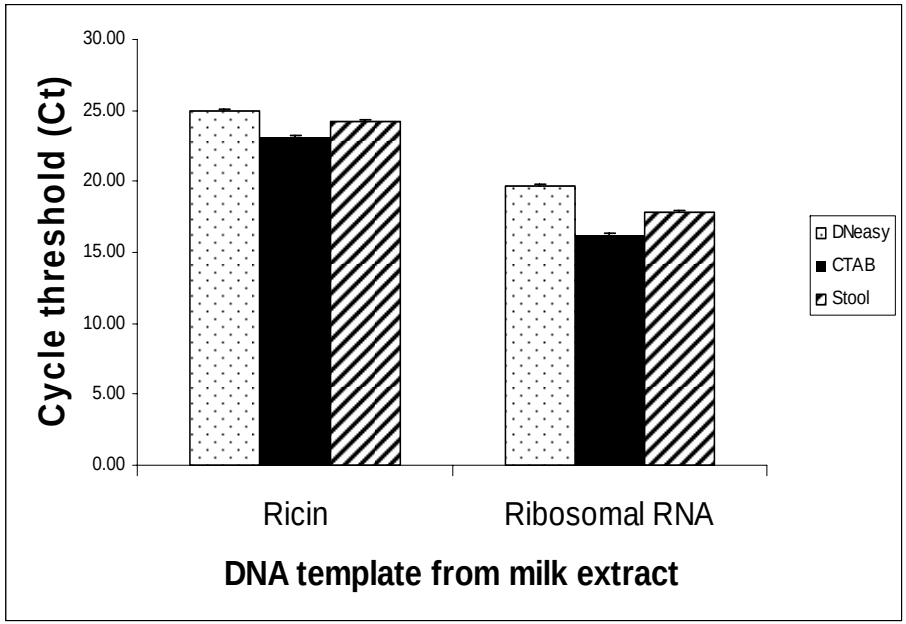
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Cycle threshold (Ct)
DNeasy
CTAB
Stool
Ricin
Ribosomal RNA
DNA template from milk extract

Fig. 3

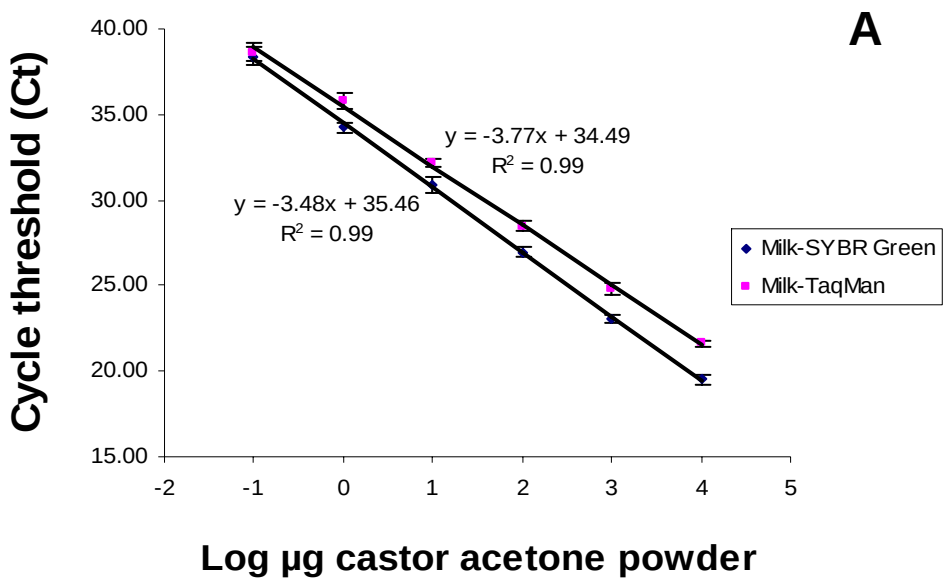
A
Cycle threshold (Ct)
40.00
35.00
30.00
25.00
20.00
15.00
-2
-1
0
1
2
3
4
5
y = -3.77x + 34.49
R2 = 0.99
y = -3.48x + 35.46
R2 = 0.99
Milk-SYBR Green
Milk-TaqMan
Log µg castor acetone powder

Fig. 4A

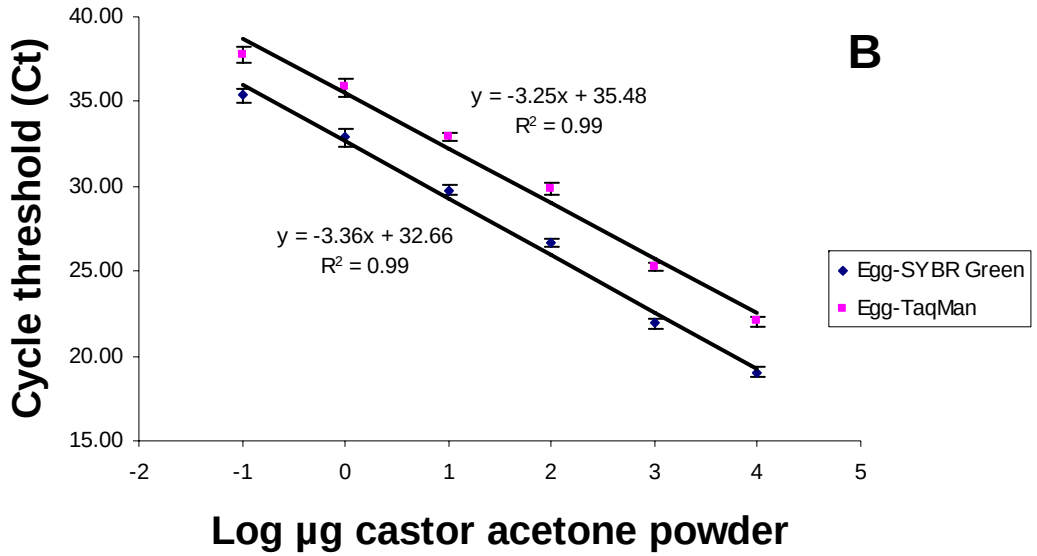
B
Cycle threshold (Ct)
40.00
35.00
30.00
25.00
20.00
15.00
-2
-1
0
1
2
3
4
5
y = -3.25x + 35.48
R2 = 0.99
y = -3.36x + 32.66
R2 = 0.99
Egg-SYBR Green
Egg-TaqMan
Log µg castor acetone powder

Fig. 4B