Vitsab User Manual 2011

L5-8 Smart Labels for Time–Temperature Indication

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Introduction

Vitsab© L5-8 Smart Labels

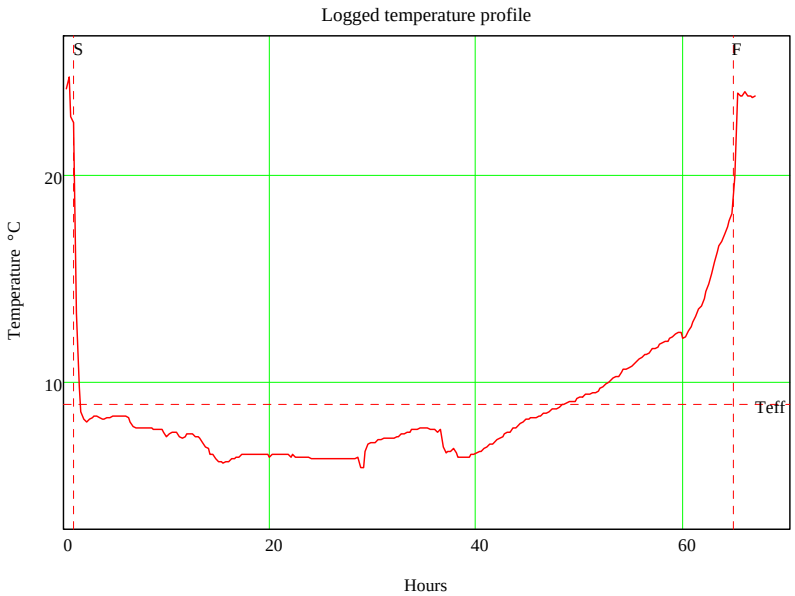
Smart labels are time–temperature indicators (TTIs) that have long been recognized by the scientific community as a simple and highly efficient tool for monitoring temperatures during distribution of perishable items. This simple low-cost biotechnology integrates temperature logging and alarm functions. TTIs monitor the cold chain temperatures of food products, for example, as they leave the controlled environment of the production/processing plant. Once activated, smart labels respond to temperature changes in real time and so give an accurate indication of the overall temperature exposure of such products over a set period — a significant advance over the general guidelines from food and health authorities to monitor the time the product spends above a specified maximum temperature. The label's visual appearance informs food-industry workers and end users whether proper refrigeration has been maintained throughout the distribution chain by reflecting the actual temperature history and — most important — whether the product is safe to eat.

Handling and Storage

Monitoring of Smart Labels during Shipment

The TTI technology of Vitsab© L5-8 labels depends on biochemical components, so labels are normally delivered by courier to ensure that their temperature is controlled during shipment. They are packaged by Vitsab in insulated boxes with cooled and thermally active gel packs, together with a temperature logger to keep track of any variations in temperature during transit.

The enclosed figure shows an example of a temperature profile (solid red line) recorded by a logger during of a smart label shipment. In this case the transport lasted 64



hours from Vitsab in Sweden to a customer in Japan. **S** represents the logger's start time and temperature when the labels were packed for shipment; **F** represents the point when package was opened by the customer at its destination. The effective temperature, $\mathbf{T_{eff}}$ (dashed line), or 'weighted-average' temperature, inside the package is calculated by the logger as 9°C. Note that even though the temperature during the last 8–10 hours of shipment rose to between 10–20°C, T_{eff} was still no more than 9°C for the full duration of the shipment.

On arrival to the end-user a marker on the logger is pressed and the logger is returned to Vitsab in the included addressed envelope so that time and temperature conditions during shipment can be evaluated and calculate; T_{eff} and the corresponding Mean Kinetic Temperature for the complete shipment. Vitsab instructions are that labels must not be used until "Certificate of Approved Delivery" has been issued by Vitsab.

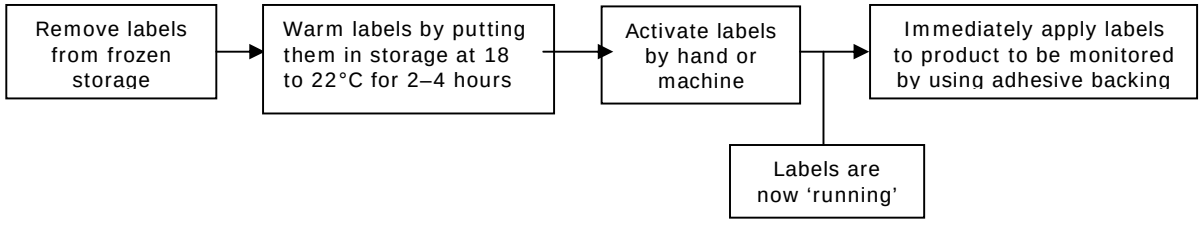
For further information regarding temperature tolerance of Vitsab© L5-8 labels prior to activation and how their response might be affected at elevated and extreme temperatures, please refer to Vitsab's Scientific Supplement 2008, which is available on request.

Storage of Smart Labels

Vitsab specify storage temperatures for Vitsab© L5-8 labels within the normal refrigerated and freezer ranges. For storage temperatures between 0–5°C the specified TTI function is guaranteed for up to 2 months and if the labels are kept frozen below –15°C up to 6 months, as indicated in the Certificate of Conformance included with each shipment. Once removed from the specified storage conditions, labels should be used immediately.

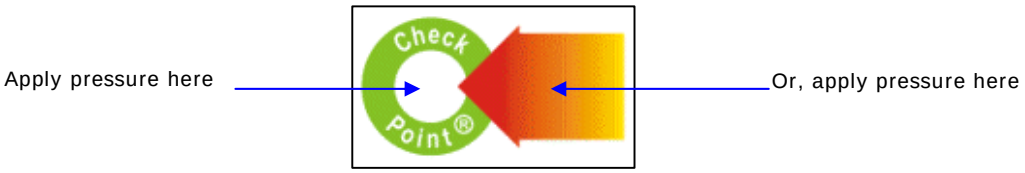
Instructions for Use: Activation and Application

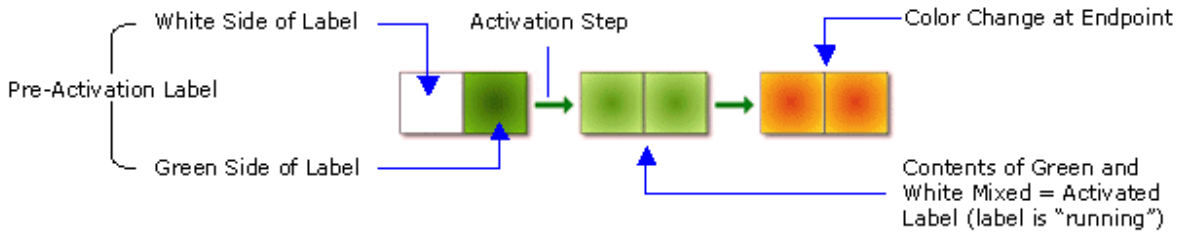
Vitsab® smart labels are supplied in a 'non-running', mode, so they must be activated prior to use according to the following simple sequence:



Every Vitsab© L5-8 label has a transparent window that displays the color of the chemical reaction taking place inside the label. Before the labels are activated, the window appears white in color. This is because the window is showing the contents of only one of the two pouches containing the reactants that make up the pre-activated label. Activation causes the reactants to combine together (see diagrams below).

Unless instructions indicate otherwise, Vitsab© L5-8 labels are activated by applying top-down pressure to the white window side of the label or to the 'arrow' side of the label (top figure). After activation of the label, the window that originally showed white (before activation) will show green. This is because the two pouches, one containing green 'component' and one containing the white 'components' are mixed together—the result is a lighter green color of the combined reactants; eventually an endpoint orange-red colour develops





Mixing of the contents is achieved by massaging the label so that the fluid contents slosh back and forth a few times to ensure proper combination. This mixing process can be performed by hand or by activation-applicator machinery. Details of an appropriate tool for this step are available from Vitsab.

The label adhesives are specially designed to operate successfully at low temperatures. However, adhesion may be weakened if the surface is wet, dirty or waxy

Instructions for Use: Detecting Endpoints

Vitsab© L5-8 labels show a defined and consistently reproducible sequence of color development during time–temperature monitoring. The green colour denoting activation becomes a clear yellow near the endpoint, finally showing as a defined orange-red at the endpoint (see figure). Endpoints can also be quantitatively validated using an appropriate colour measuring laboratory instrument.

1 	Standby, or non-running, label. The white central window means that the label has not yet been activated.
2 	Running, activated label, with green central window. This is how the label looks immediately after activation and for most of the time–temperature monitoring period.
3 	Just before the endpoint, the central window changes to a yellowish color. Intermediate steps manifest as a yellowish green.
4 	The endpoint is reached when the central window first turns orange-red in color. Once established, the label has passed the endpoint.

For practical purposes, we have set the endpoint at the start of the yellow to orange–red color transition (that is, as shown between panels 3 and 4 above), when users are alerted by a notification on the label such as “Do not use” or “Expired” (the exact message depends on the requirements of the customer who purchases labels for specific product applications).

Stages 3 and 4 occur rapidly, comprising only a small fraction of the label’s total running time. The above images bracket the subjective endpoint required by the user.

Smart Labels and Food Safety

Our smart labels are widely used for monitoring the chill chain condition of for example seafood products. They are formulated to produce a response that conforms to the established guidelines for HACCP regulations for packaging seafood under reduced-oxygen and modified-atmosphere conditions.

Using Smart Labels for *Clostridium botulinum* Control

The following is an excerpt from a document produced in January 1999 by the U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, 'HACCP REGULATION FOR FISH AND FISHERY PRODUCTS — QUESTIONS AND ANSWERS':

> **"Question:** What are the factors that make *Clostridium botulinum* a hazard that is reasonably likely to occur in a fishery product?
>
> **Answer:** Some factors which contribute to the likelihood of a *Clostridium botulinum* hazard include packing the product under vacuum, in a deliberately modified atmosphere, in a hermetically sealed container, inside a package with a film covering that restricts inward oxygen diffusion, or in oil. Historically, the hazard been controlled by applying a moderate heat treatment in combination with salt at less that 10%, or other reduced water activity methods. This type of processing is not currently in widespread use due to consumer preferences for minimally processed fresh products.
>
> For additional information, consult FDA's 'Fish and Fishery Products: Hazards and Controls Guide' (Guide), Edition Two, p. 154.
>
> **Question:** Is *Clostridium botulinum* (C. bot.) a hazard in vacuum packaged raw seafood products that are stored and distributed refrigerated?
>
> **Answer:** Yes, the Guide states that FDA is not aware of any suitable controls for C. bot. in vacuum packaged raw fish, i.e. such a product would not contain any known barriers to the growth of C. bot. Refrigeration alone is not a suitable barrier without adequate temperature control (monitoring) from processor to consumer. If a processor intends to pack raw fish in a vacuum package, he will need to establish adequate safety controls. The most likely procedure would be to carry out inoculated pack studies."

The key phrase here is **"*adequate temperature control (monitoring)*"**, but what does this term actually mean in practice?

In reply to this question, consider an excerpt from another document produced in January 1998 by the U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition 'FISH AND FISHERY PRODUCTS HAZARDS AND CONTROLS GUIDE' — Chapter 13, '*CLOSTRIDIUM BOTULINUM* TOXIN FORMATION':
"If you intend to vacuum package, or use modified atmosphere packaging or hermetically sealed packaging for these products or pack them in oil or a similar oxygen excluding media, you will need to evaluate the effectiveness of other preventive measures, either singularly, or in combination. Such evaluation will usually necessitate the performance of inoculated pack studies under moderate abuse conditions. An example of another preventive measure to consider is strict temperature controls throughout distribution and retail sale, such as the use of recorder thermometer charts or digital time/ temperature data loggers during distribution and retail storage and sales, or time/temperature integrators on individual packages."

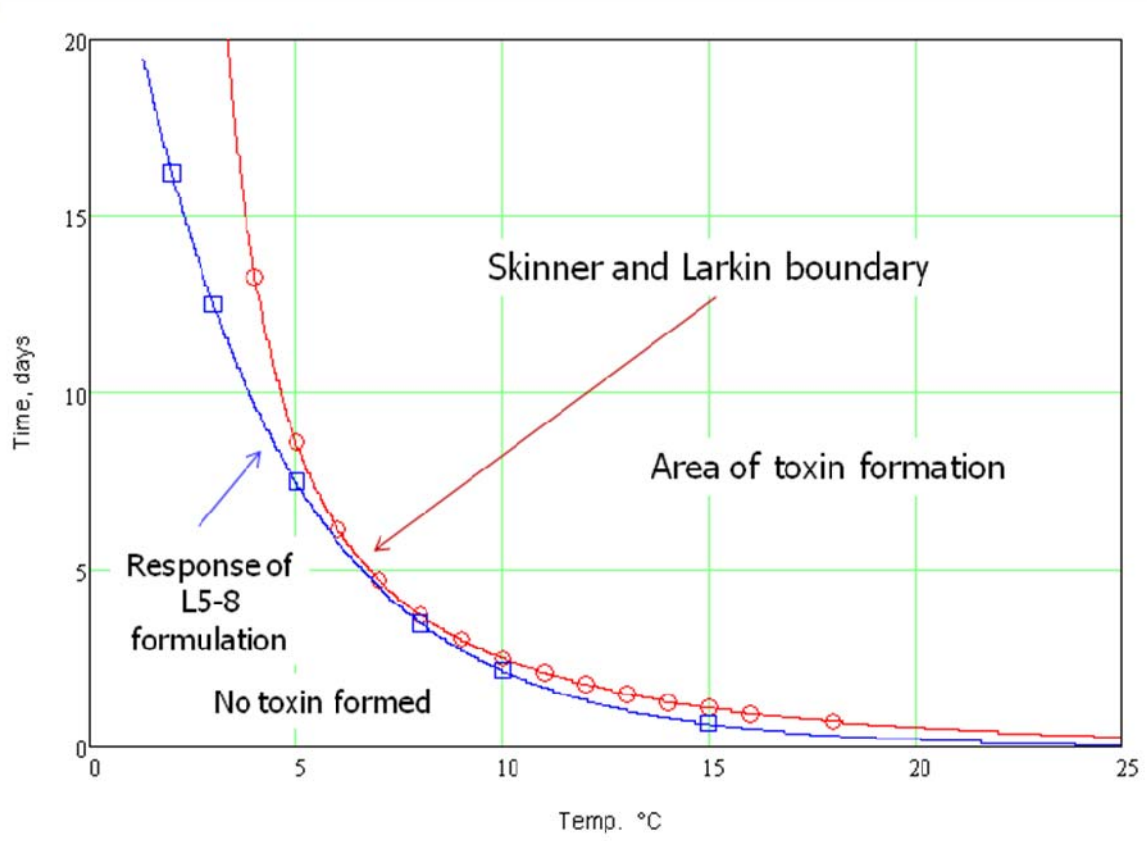
It is not cost-effective to employ recorder thermometer charts or digital time/temperature data loggers for the entire distribution scheme from processor to consumer for seafood packaged under reduced-oxygen conditions. Time–temperature integrators are recognized as being the most

effective and cost-effective tool for guarding against the production of botulinum toxin in food products (for a scientific rationale, see G. E. Skinner and J. W. Larkin's paper 'Conservative prediction of time to *Clostridium botulinum* toxin formation for use with time–temperature indicators to ensure safety of foods' in *Journal of Food Protection* 61, 1154–1160; 1998).

The Vitsab© L5-8 label's TTI formulation has been developed to conform to the Skinner–Larkin model (see Vitsab's Scientific Supplement Sep 2008 for details) and the FDA's HACCP regulations.

The figure below shows the time to endpoint-color development as a function of temperature for a Smart Label TTI (blue line). The red line shows the Skinner–Larkin boundary curve, which describes the threshold of toxin formation as a function of temperature. The implications of the diagram are that sets of time and temperature conditions below and to the left of the Skinner–Larkin boundary line there is no toxin formed.

L5-8 response and Skinner and Larkin boundary curve.



Test labels were run in controlled temperature cabinets to determine the time-temperature (TT) response of the L5-8 label formulation. The red line and red circles represent the Skinner–Larkin borderline function for incipient *Clostridium botulinum* toxin formation. The blue line represents an exact Arrhenius curve fitted to the experimental L5-8 data (blue squares).
It can be seen that the response of the L5-8 label corresponds well to the Skinner–Larkin boundary and thus to the contour of no *Clostridium botulinum* toxin formation, as described by Skinner and

Larkin. Vitsab L5-8 labels are thus an appropriate application as a smart label control instrument, since the endpoint of the label response creates a warning signal prior to approaching Skinner and Larkin boundary time–temperature values through a change to an orange-red color.

In conclusion the L5-8 label therefore acts as a functional HACCP control that encompasses the lower threshold limits for the toxin effect.

Validation of the Vitsab® L5-8 label

L 5-8 Time–Temperature Response

Prior testing has established the information shown in the table about the vulnerability of the labels to environmental factors. We periodically repeat all of these tests for L5-8 labels and other formulations for environmental susceptibility and conformity to manufacturing specifications.

Environmental Factor	**Susceptibility**
Carbon dioxide	Acceleration of timing by less than 5% if left in 100% CO_2 for more than 24 hours. Lower CO_2 concentrations produce negligible effects on label time-to-endpoint characteristics. Evidence indicates that acceleration effects are completely reversible if the time-run of a specific label is long enough for outward diffusion to reset the chemical reaction of endpoint color production.
Sunlight/UV Radiation	No effect when parallel sample groups are tested.
Carbon monoxide)	No effect when parallel sample groups are tested.
Ionizing Radiation	No effect when parallel sample groups are tested by exposure to soft beta radiation. Not susceptible to normal food irradiation levels.
Anaerobic Conditions	No effect when parallel sample groups are tested.
Storage Temperature	Refer to shipping and storage guideline information above.

Vitsab International has performed exhaustive validation testing of its L 5-8 formulation. A number of labels were tested according to the type of time-temperature function described for *Clostridium*

botulinum thresholds for toxin formation as previously indicated. Please refer to Vitsab Scientific Supplement Sep 2008 for more information

Process Controls Used in Vitsab Manufacturing

All materials used in the manufacture of L5-8 labels are subject to inspection and testing. These tests are summarized in our scientific supplement: *Vitsab L5-8 Sci-808*, which is available on request.

All production runs are indexed with a batch number giving full traceability. Post-production label batches are kept in frozen storage for all shipped product for up to a year after shipment. Periodic tests of TT performance are made to verify proper function. Data-based records are kept for all verification and quality-control tests, which are performed in-house. The manufacturer of the label's supporting film also maintains records of film-production tests.

Vitsab recommendations

With the analysis of logger data Vitsab validate each individual shipment to customers. Thus for shipments to customers Vitsab advise the following:

Effective temperature (Teff)	Duration of shipment	Shipment status
Less than 15°C	Shorter than 10 days	OK
15 - 20°C	Shorter than 96 hours	OK
20 - 25°C	Shorter than 48 hours	OK
25 - 35°C	Shorter than 12 hours	OK

The effective temperature is a weighted average of temperature for the duration of the shipment in literature also referred to as mean kinetic temperature (MKT).

As have been pointed out previously L5-8 labels should be stored either chilled or frozen the reason being that label function is based on an enzyme reaction and as most biological material should be stored chilled or frozen to maintain its set activity. Vitsab advise the following:

Storage temperature	Storage time
-20 to -15°C	6 months
0 to +5°C	2 months

If customers after an extended time of storage need to verify the preset function of the label a simple validation test can be carried out. A label or a set of 5 – 10 labels are brought out of storage allowed to temperature equilibrate to room temperature around 20°C e.g. overnight, then activated and put in a constant temperature and monitor the response time. The response time should be within the given range in the table below.

Constant temperature	Response time
10°C	2.5 days ±10%
15°C	24 hours ±10%
20°C	8.5 hours ±10%

The variation in response time can be due to how well activation has been accomplished i.e. mixing or massaging the contents of the two pouches during the activation procedure.

Using the above recommended procedures customers will be sure of fully viable L5-8 labels when put to use.

Appendices

Appendix A

Endpoint-Setting Processes

Subjective Reading

Vitsab L5-8 labels indicate a defined process endpoint for perishables of different kinds. And it can be based on the time-temperature characteristics of that process. The endpoint is signaled by a change in color of the target dot of the label. Personnel interpreting the label use a subjective judgment of color to determine if the endpoint of the defined process has been reached. This color decision is then used in product handling, acceptance or rejection, or for other purposes.

The current color system of the labels will be transitioning from an initial green to a yellow → greenish orange → orange-red color. Some panelists see the final color transition to "red", others see it a transition to "orange". This final transition comprises only a small fraction of the entire run time of a L5-8 label.

Panellists were asked to determine endpoints based on "first appearance of orange-red color". The panel participants were shown a graded series of color development stages of the labels, and a specific color endpoint was determined by the preponderance of selections of endpoint. Panel results were almost unanimous in the selection.

Appendix B – General Validation of Vitsab© L5-8 Labels

A thorough validation review for Vitsab technology can be found in our scientific supplement *Vitsab Scientific Supplement Sep 2008*, which is available on request.

Appendix C – Material Safety Data Sheet for Vitsab© L5-8 Labels.

Section 1 - Product Identification

Product number	Application	Product Type
L-Series Labels	Perishable Foods	Single dot

Section 2 - Information on Ingredients

Chemical Name	CAS No.
Albumin, Bovine	9048-46-8
CHES Buffer	103-47-9
Glycerol	56-81-5
PVC	9002-86-2
Trilaurin	37318-95-9
Methyl Myristate	124-10-7
Xanthan Gum	11138-66-2
Polypropylene, Plastic film	
Distilled Water	
Lipase	
pH range = 8.9 - 5.5	

Section 3 - Physical and Chemical Characteristics

Appearance: Plastic film with mini-pouches containing partly water-soluble components.

Properties of the plastic film:

Boiling point: ND
Specific gravity: ND
Melting point: 130 °C

Solubility in Water: None
Reactivity in Water: None
Vapor Pressure (mm Hg): ND

Properties of the contents of mini-pouches:

Boiling point: ND
Specific gravity: 1.11 at 68°F (20°C)
Melting point: < -13°F (- 25°C)

Solubility in Water: 99 %
Reactivity in Water: None
Vapor Pressure (mm Hg): < 1 at 68°F (20°C)

Section 4 - Fire and Explosion Data

The plastic laminate will burn under the right conditions of heat and oxygen supply. Fires can be extinguished by conventional means, with water fog preferred. As with any fire use self-contained breathing apparatus with facial mask to avoid inhalation of hazardous combustion fumes. Fumes may contain hydrocarbons and oxides of carbon, such as carbon monoxide, carbon dioxide as well as water.

The content of the aqueous solution in the pouch can also generate fumes containing hydrocarbons and oxides of carbon, such as Carbon Monoxide, Carbon Dioxide as well as water and Hydrogen Chloride.

Section 5 - Reactivity Data

The plastic laminate as well as the pouch content is stable i.e. the whole product is stable.

Incompatibility (materials to avoid):	Strong Oxidizing Compounds, Strong Acids, and Strong Bases
Hazardous Polymerization:	Will Not Occur
Hazardous Decomposition Products:	When exposed to intense heat resulting in decomposition the product may release toxic fumes such as Acrolein, Carbon Monoxide, Carbon Dioxide and Hydrogen Chloride.

Section 6 - Health Hazards

Threshold Limit Value (TLV):	NA
Effects of Overexposure:	None
Emergency First Aid Procedures:	Skin and Eyes: In the event of rupture of the pouch, flush immediately with copious amounts of water. Get medical attention for eyes. Wash skin with soap and water.

Initial pH of pouch content is 8.9 after activation of TTI while TTI color is green. This drops to about 5.5 when TTI has changed color to red-orange.

NA = Not Applicable **ND = Not Determined**