Interpretation of Microbiological Test Results

Nicola Elviss
FW&E Microbiology Network
June 2010
PHLS Guidelines for the microbiological quality of some ready-to-eat foods at the point of sale: 1992, revised 1996 and 2000

Communicable Disease and Public Health

Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale

A working group (RJ Gilbert, J de Louvois, T Donovan, C Little, K Nye, CD Ribeiro, J Richards, D Roberts, FJ Bolton) of the PHLS Advisory Committee for Food and Dairy Products

pp163-7
Revision of the PHLS Guidelines: 2009

Need for interpretation of results from single samples collected during:

- Predefined surveys
- Food inspections
- Follow-up from adverse findings or complaints
- Investigation of outbreaks

Should serve to compliment EU regulations and criteria
HPA Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market

Issued November 2009

Available from: http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1259151921557
These guidelines do not take precedence over microbiological criteria within European or national legislation but serve to complement legally enforceable standards and provide an indication of the microbiological safety of foods where standards currently do not exist.
HPA Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market: 2009

These guidelines are for use by Food Examiners and enforcement officers in identifying situations requiring investigation for public health or food safety reasons.
Pathogens in Foods Placed on the Market

Hazard

Result in 25g

Risk Category

Interpretation

Likely cause

Suggested action

Laboratory specialist and reference tests
Interpretation used in HPA Ready-to-eat Guidelines: 2009

The terms used to express the microbiological quality and/or safety of the ready-to-eat foods are:

**Satisfactory**
Test results indicating good microbiological quality

**Borderline**
Test results that are not satisfactory or unsatisfactory, are at the upper limits of acceptability and which indicate the potential for development of public health problems and of unacceptable risk.
**UNSATISFACTORY/Potentially injurious**

Pathogens

Test results indicate a product that is potentially injurious to health and/or unfit for human consumption and require immediate remedial action.

Such results may be part of prosecutions by environmental health department, especially if they occur in more than one sample.

**UNSATISFACTORY**

Hygiene indicator

Test results that require remedial action.

Prosecutions based solely on aerobic colony counts or indicator organisms, even when unsatisfactory, is unlikely to be successful.
Examples of guidance on the interpretation of results for detection of pathogens (the hazard) in ready-to-eat foods placed on the market: 2009

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Result/25g</th>
<th>Risk Category</th>
<th>Interpretation</th>
<th>Likely cause</th>
<th>Suggested action</th>
<th>Laboratory specialist and reference tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em> spp. (thermotolerant)</td>
<td>Detected</td>
<td>High</td>
<td><strong>UNSATISFACTORY and Potentially injurious to health and/or unfit for human consumption</strong></td>
<td>Inadequate processing Cross contamination</td>
<td>Immediate investigation of: the food origin, production process and environment; take investigative food samples and consider environmental monitoring.</td>
<td>Confirmation of identity, molecular typing.</td>
</tr>
<tr>
<td></td>
<td>Not detected</td>
<td>Low</td>
<td><strong>SATISFACTORY</strong></td>
<td></td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>
Examples of guidance on the interpretation of results for detection of pathogens (the hazard) in ready-to-eat foods placed on the market: 2009

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<tr>
<td><em>Salmonella</em> spp.</td>
<td>Detected</td>
<td>High</td>
<td>UNSATISFACTORY and Potentially injurious to health and/or unfit for human consumption</td>
<td>Inadequate processing Cross contamination</td>
<td>Immediate investigation of: the food origin, production process and environment; take investigative food samples and consider environmental monitoring.</td>
<td>Confirmation of identity, serotyping, phage typing, anti-microbial resistance patterns, molecular typing</td>
</tr>
<tr>
<td></td>
<td>Not detected</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td>N/A</td>
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### Examples of guidance on the interpretation of results for enumeration of pathogens (the hazard) in ready-to-eat foods placed on the market: 2009

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<tr>
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<tbody>
<tr>
<td>Listeria monocytogenes</td>
<td>&gt;10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>High</td>
<td>UNSATISFACTORY: Potentially injurious to health and/or unfit for human consumption</td>
<td>Strong evidence for poor processing, environmental contamination, cross contamination during processing or at point of sale, poor temperature control, or inappropriate length of shelf life</td>
<td>Immediate investigation of: the food origin, production process and environment; take investigative samples of food and environmental monitoring.</td>
<td>Refer isolates for confirmation of identity, serotyping, molecular typing</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;=&lt;/sup&gt;10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Moderate</td>
<td>BORDERLINE</td>
<td>Likely evidence for poor processing and/or poor quality raw materials</td>
<td>Risk will increase proportional to the levels detected and the likelihood of subsequent growth under normal storage conditions. Review quality of raw materials, food preparation environment (including cleaning), cooking, temperature and shelf life controls. Consider re-taking investigative sampling food and environmental monitoring. In long shelf life foods where there is potential for growth during storage, and in foods likely to be served to vulnerable groups (such as that served in hospital) the presence of L. monocytogenes at any level may be significant and should be investigated.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td>N/A</td>
<td>Consider referral of isolates, particularly where associated with persistent contamination or as part of outbreak investigations. For foods in high risk categories, refer all isolates for reference testing.</td>
</tr>
</tbody>
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Examples of guidance on the interpretation of results for enumeration of pathogens (the hazard) in ready-to-eat foods placed on the market: 2009

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<tbody>
<tr>
<td><em>Staphylococcus aureus</em> and other coagulase positive staphylococci</td>
<td>&gt;10⁴</td>
<td>High</td>
<td>UNSATISFACTORY: Potentially injurious To health and/or Unfit for human consumption</td>
<td>Strong evidence for poor handling and temperature control.</td>
<td>Immediately review food handling as well as temperature and time controls. Take investigative sample of food, food preparation environment and food handlers.</td>
<td>Not all strains are capable of producing toxin and causing disease. Confirmation of identity, typing pathogenicity (toxin gene detection) of isolates. Consider enterotoxin detection in food and food remnants from cases of suspect food poisoning or where high levels (&gt;10⁵ cfu/g) may have occurred at any stage in the food chain</td>
</tr>
<tr>
<td></td>
<td>20 – 10⁴</td>
<td>Moderate</td>
<td>BORDERLINE</td>
<td>Likely evidence for poor handling, process and temperature control.</td>
<td>Risk will increase proportional to the levels detected and the likelihood of subsequent growth in the absence of appropriate levels of control. Review handling as well as processing controls, especially if there opportunities for growth of staphylococci during processing or maturation of the product. Consider taking investigative samples of food, food preparation environment and food handlers.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;20</td>
<td>Low</td>
<td>SATISFACTORY</td>
<td></td>
<td>N/A</td>
<td>Consider referral of isolates, particularly where associated with outbreak investigations or where there is die off of the bacterium during storage</td>
</tr>
</tbody>
</table>
Examples of guidance on the interpretation of results for hygiene indicator organisms in ready-to-eat foods placed on the market: 2009

<table>
<thead>
<tr>
<th>Hygiene Indicator</th>
<th>Results (cfu/g)</th>
<th>Interpretation</th>
<th>Comment</th>
<th>Likely cause</th>
<th>Suggested action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>&gt;10⁴</td>
<td>UNSATISFACTORY</td>
<td>Members of this group occur in the environment as well as the gut of man and animals. Their presence at these levels suggests an overall poor general hygienic status of a food product. These bacteria are not reliable indicators of contamination by faecal pathogens in a food.</td>
<td>Poor hygiene due to undercooking, or cross contamination from raw meat, staff or food contact surfaces as well as poor temperature and time control.</td>
<td>Review cooking and all hygiene procedures including cleaning. Take investigative samples of food and undertake environmental monitoring of food preparation environment.</td>
</tr>
<tr>
<td></td>
<td>10² - ≤10⁴</td>
<td>BORDERLINE</td>
<td>Interpret in conjunction with test results from other microbiological parameters but detection in several foods or other areas of the food production environment should be investigated.</td>
<td>Possible evidence of poor hygiene due to undercooking, or cross contamination from raw meat, staff or food contact surfaces as well as poor temperature and time control.</td>
<td>Review cooking and all hygiene procedures including cleaning. Consider taking investigative samples of food and the food preparation environment. Action should be proportional to levels detected.</td>
</tr>
<tr>
<td></td>
<td>&lt;10²</td>
<td>SATISFACTORY</td>
<td></td>
<td>N/A</td>
<td>None</td>
</tr>
</tbody>
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### Examples of guidance on the interpretation of results for hygiene indicator organisms in ready-to-eat foods placed on the market: 2009

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<thead>
<tr>
<th>Hygiene Indicator</th>
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<th>Interpretation</th>
<th>Comment</th>
<th>Likely cause</th>
<th>Suggested action</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>&gt;10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>UNSATISFACTORY</td>
<td>Originates from the intestinal tract of man and animals indicating contamination and growth (depending on the level detected) at some stage of the process. The detection of <em>E. coli</em> is not a reliable indicator that faecal pathogens are present in the food and results should be interpreted in conjunction with test results from other microbiological parameters. Repeated or widespread detection in several foods or environmental sites highlights an increased food safety risk.</td>
<td>Poor hygiene due to undercooking, or cross contamination from raw food especially meat, staff or food contact surfaces as well as poor temperature and time control.</td>
<td>Review cooking and all hygiene procedures including cleaning. Take investigative samples of food and undertake environmental monitoring of food preparation environment.</td>
</tr>
<tr>
<td></td>
<td>20 - ≤10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>BORDERLINE</td>
<td>Although <em>E. coli</em> should not be detected in ready-to-eat foods, low levels may occasionally be found. Repeated or widespread detection in several foods or areas of the food production environment suggests an increased food safety risk.</td>
<td>Possible evidence of poor hygiene due to undercooking, or cross contamination from raw food especially meat, staff or food contact surfaces, as well as poor temperature and time control.</td>
<td>Review cooking and all hygiene procedures including cleaning. Consider taking investigative samples of food and the food preparation environment. Action should be proportional to levels detected.</td>
</tr>
<tr>
<td></td>
<td>&lt;20</td>
<td>SATISFACTORY</td>
<td>N/A</td>
<td>N/A</td>
<td>None</td>
</tr>
</tbody>
</table>
HPA Ready-to-eat Guidelines: 2009
Aerobic Colony Counts

13 food categories
Categorised based on processes used during preparation and storage.

Category 1-9
ACC result can give useful information about the microbiological quality of food.

Category 10-13
ACC not routinely performed but may be useful if investigating spoilage.
Aerobic Colony Counts
Food Categories 1-9: (Satisfactory)

1. Ambient stable, canned, bottled, carton, pouched (<10)
2. Recently cooked (<10³)
3. Cooked chilled minimum handling (<10⁴)
4. Bakery (no cream), powdered foods after reconst’n (<10⁴)
5. Cooked chilled some handling (<10⁵)
6. Non-fermented dairy products, mayonnaise, cooked sauces (<10⁵)
7. Food mixed with dressings and dips (<10⁶)
8. Extended shelf-life refrigerated products (<10⁶)
9. Raw RTE meat/fish, cold smoked fish (<10⁶)
Food Categories 10-13:

ACC test not applicable

10. Pickled, Marinated, salted
11. Dried foods
12. Fresh fruit and vegetables or any product containing these products
13. Fermented meat and vegetables, cured and dried meats, ripened cheese
Interpretation of ACC results based on HPA RTE Guidelines: 2009

**Satisfactory**

No action required

**Borderline**

Consider source of food and stage in shelf life.
Further investigate if other samples from the same source are also borderline

**Unsatisfactory**

Investigate
Microbiology results will assist with a risk assessment process

<table>
<thead>
<tr>
<th>Hazard identification</th>
<th>What will do the harm?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazard characterisation</td>
<td>What will the effect be?</td>
</tr>
<tr>
<td>Exposure assessment</td>
<td>How much of the hazard will be eaten?</td>
</tr>
<tr>
<td>Risk characterisation</td>
<td>How likely is it that harm will be done and how severe will this harm be?</td>
</tr>
</tbody>
</table>
1. **Chicken roll (no salad) : Sandwich bar (routine inspection)**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic Colony Count</td>
<td>$9 \times 10^5$ cfu/g</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>$3 \times 10^5$ cfu/g*</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>$6 \times 10^3$ cfu/g*</td>
</tr>
<tr>
<td>Listeria spp. (total)</td>
<td>$&lt;10$ cfu/g</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>$&lt;20$ cfu/g</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Not detected in 25g</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>Not detected in 25g</td>
</tr>
</tbody>
</table>

**UNSATISFACTORY based on HPA RTE Guidelines: 2009**

Unsatisfactory due to *Enterobacteriaceae* and *Escherichia coli* counts.

Note: if this sample contained salad ACC and Enterobacteriaceae tests would not have been performed.
1. Likely causes

Poor hygiene due to undercooking, or cross contamination from raw meat, food handlers or food contact surfaces as well as poor temperature and time controls.

Suggested actions (not exclusive)

Review cooking and all hygiene procedures including cleaning.

Take investigative samples of food and undertake environmental monitoring of food preparation environment.

Inspection of food preparation areas

Poor level of hygiene and cleaning
2. Homemade Beef Casserole: Cafe (routine inspection)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic Colony Count</td>
<td>&lt;1x10^3 cfu/g</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>&lt;1x10^2 cfu/g</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>&lt;20 cfu/g</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>&lt;20 cfu/g</td>
</tr>
<tr>
<td>Listeria species (total)</td>
<td>&lt;20 cfu/g</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>8x10^4 cfu/g*</td>
</tr>
<tr>
<td>Salmonella species</td>
<td>Not Detected/25g</td>
</tr>
</tbody>
</table>

**UNSATISFACTORY/ Potentially injurious to health bases on HPA RTE Guidelines : 2009**

Unsatisfactory due to *Clostridium perfringens* count.

Note: *Clostridium* species do not grow aerobically.
2. Likely cause

Strong evidence for poor processing, particularly during cooling period after cooking, the use of left over food or from stocks and gravies.

Suggested Actions (not exclusive)

Immediately review temperature and time controls. Take investigative samples of food and the food preparation environment.

Additional Information

Not all strains are capable of producing toxin and causing disease

Inspection of food preparation areas

Poor temperature and time control

Poor food preparation and storage environments

*C. perfringens isolates non-toxigenic*
3. Tuna Sandwich (no salad) : Supermarket

(associated with an outbreak of suspected Scombrotoxin poisoning)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic Colony Count</td>
<td>$3 \times 10^8$</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>$2 \times 10^7$</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>&lt;20</td>
</tr>
<tr>
<td><em>Listeria</em> spp. (total)</td>
<td>&lt;10</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>&lt;20</td>
</tr>
<tr>
<td><em>Salmonella</em> species</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

UNSATISFACTORY based on HPA RTE Guidelines: 2009

Unsatisfactory due to Aerobic Colony Count and Enterobacteriaceae count.
3. Likely causes

Poor hygiene due to undercooking, or cross contamination from raw meat, food handlers or food contact surfaces as well as poor temperature and time controls.

Suggested Actions (not exclusive)

Review cooking and all hygiene procedures including cleaning.

Take investigative samples of food and undertake environmental monitoring of food preparation environment.

Inspection of food preparation areas

Poor temperature and time control

Sandwich sent for histamine analysis, contained >200ppm
4. Vanilla Slice: Confectioners
(associated with an outbreak)

<table>
<thead>
<tr>
<th>Bacterial Group</th>
<th>Count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic Colony Count</td>
<td>$9 \times 10^5$</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>$&lt;1 \times 10^2$</td>
</tr>
<tr>
<td><em>Listeria</em> species (total)</td>
<td>$&lt;20$</td>
</tr>
<tr>
<td><em>Bacillus</em> spp. (total)</td>
<td>$3.5 \times 10^2$</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>$8 \times 10^5$</td>
</tr>
</tbody>
</table>

**UNSATISFACTORY/ Potentially injurious to health**
based on HPA RTE Guidelines: 2009

Unsatisfactory due to *Staphylococcus aureus* count.
Food sample and isolated will be referred for further analysis.
4. Likely causes

Strong evidence for poor handling and temperature control.

Suggested Actions (not exclusive)

Immediately review food handling as well as temperature and time controls. Take investigative samples of food, preparation environment and food handlers.

Additional Information
Not all strains are capable of producing toxin and causing disease.

Inspection of food preparation areas

Vanilla custard added to slices by hand

*S.aureus* contained enterotoxin genes

Enterotoxin detected in food remnants
5. Sliced Tongue: Butcher’s Shop
(routine inspection)

Aerobic Colony Count  \(2 \times 10^5\) cfu/g
Enterobacteriaceae  \(<1 \times 10^2\) cfu/g
*Escherichia coli*  40 cfu/g
*Staphylococcus aureus*  \(<20\) cfu/g
*Listeria* spp. (total)  \(4 \times 10^3\) cfu/g*
*Listeria monocytogenes*  \(4 \times 10^3\) cfu/g*
Salmonella species  Not Detected/ 25g

**USATISFACTORY/ Potentially injurious to health based on HPA RTE Guidelines: 2009**

Unsatisfactory due to *Listeria monocytogenes* count.
5. Likely cause

Strong evidence for poor processing, environmental or cross contamination during production or at point of sale, poor temperature control or inappropriate length of shelf life.

Suggested Actions (not exclusive)

Immediate investigation of the food origin, production process and environment. Take investigative samples of food and environmental monitoring.

Additional Information
Isolates should be referred for confirmation of identity and molecular typing

Inspection of food preparation areas
Re-sampling showed wider contamination of other meat products
Environmental sampling showed contamination of slicing machine
Don’t Forget Risk Communication