final report

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Prepared by: Symbio Alliance
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Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Selection and use of ATP Machine for hygiene monitoring in Australian meat processing plants

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CONTENTS

1. PURPOSE OF DOCUMENT

The purpose of these guidelines is to assist Australian meat processing establishments with the consistent application of ATP measurement and use of ATP results. The

2. WHAT IS ATP AND HOW MIGHT ITS MEASUREMENT BENEFIT A MEAT PROCESSOR?

An effective sanitation program is critical to the quality and safety of manufactured food products. Food processing companies are well aware of the importance and consequences of poor hygiene. Poor cleaning could result in contamination of the final product from unclean environment, construction, equipment or personnel.

For most food processors, poor hygiene presents an unacceptable risk. Testing cleaning effectiveness is increasingly important especially with increased concerns over cross contamination with allergen residues. Consumer health, company revenue and brand value could easily be put in jeopardy by a food poisoning outbreak or consumer level product recall. It is for these reasons, in combination with such things as meeting regulatory and customer specific requirements that control over the hygiene of the processing environment is so important.

2.1 TRADITIONAL METHODS OF HYGIENE TESTING

Currently the Australian meat processing industry uses a combination of a visual appraisal of cleanliness and microbiological verification for pre-operational hygiene checks.

The visual appraisal is immediate but is subjective and not very sensitive - a surface which looks clean may have microbes present and/or biofilms which could harbour bacteria.

Traditional microbiological testing is more objective but only considers bacteria present, not biofilms without bacteria. It is performed by taking and culturing microbial samples, such as aerobic plate counts (APC). The long delay in obtaining results from microbial testing (at least 48 hours) makes it impossible to undertake timely corrective action.

Thus the current system could be considered too slow, lacking in objectivity and may not detect product residues invisible to the naked eye.

2.2 WHAT IS ATP?

ATP stands for adenosine-5'-triphosphate. ATP consists of adenosine ─ composed of an adenine ring and a ribose sugar ─ and three phosphate groups (triphosphate). The importance of ATP is that it is the basic energy currency molecule for all types of living organisms and as such is present in all microorganisms, plant and animal cells. The ability to detect ATP to identify residues of foods and microorganisms can be extremely useful in determining the effectiveness of a cleaning program.

Rather than ascertaining the number of microorganisms as an indicator of cleanliness as currently performed in the meat processing industries, the approach of using ATP is to ascertain the levels of biological residues. Even though food residue may not be inherently dangerous, its presence indicates that a surface has not been thoroughly cleaned and may either harbour pathogenic organisms or provide a medium for their subsequent growth. Using food residue as an indicator for cleanliness is a similar idea to the use of generic E. coli to assess the level of faecal contamination.

Technologies based on ATP bioluminescence have been developed to measure Adenosine Tri-Phosphate (ATP) and provide an immediate, objective and accurate result. It is proposed that when used in conjunction with targeted microbiological testing, ATP measurement technologies can greatly enhance the effectiveness of verification of plant and equipment hygiene.

2.3 HOW ATP IS USED IN OTHER SECTORS OF THE FOOD INDUSTRY

ATP bioluminescence is currently used widely in the food industry both domestically and internationally. The use of ATP bioluminescence in Australia is currently dominated by the dairy industry. ATP tends to be used in conjunction with traditional microbiological methods such as Aerobic Plate Counts (APC). ATP tests are often taken on product contact surfaces and used as an immediate verification of the cleaning program thus the suitability to positive release of lines for production. On average, 15 - 20 ATP bioluminescence swabs may typically be taken per day as part of preoperational inspection, depending on the complexity of the process and extent of equipment and surfaces being cleaned.

Companies utilizing ATP bioluminescence generally establish Pass/Fail or Pass/Caution/Fail limits by validating the measurements from the ATP machine against visual and microbiological results. Some operators have set generic pass/caution/fail limits for all types of surfaces, while others have set specific pass/caution/fail limits for individual test points based on past history of results. The limits set for any point can be linked to the risk involved, the type of surface and may be incrementally decreased over time in order to improve overall hygiene of plant. Operators have noted that after implementing ATP bioluminescence testing, they have seen a reduction in routine environmental and product microbiological test failures.

It has been reported that companies utilise data trending software as an aid to: assess the effectiveness of the cleaning teams; set benchmarks; identify abnormal cleaning events; improve the cleaning program; identify trends in procedures and surfaces; and create reports for audit.

There is no evidence of ATP bioluminescence being used in red meat processing establishments in Australia and no published evidence of this technology being utilized in red meat abattoirs in the USA and Brazil. While it is reported that in the United Kingdom, 88% of red meat abattoirs surveyed did not use ATP bioluminescence for environmental monitoring, of those that did use the technology, they did not record the results.

2.4 HOW ATP CAN IMPROVE HYGIENE IN THE RED MEAT INDUSTRY

ATP bioluminescence has benefits such as immediate quantifiable results, data gathering and tracking software, ease of use and cost effectiveness. If the Australian red meat industry implemented the use of ATP bioluminescence, it is believed the industry could assume many benefits already realised by the dairy industry**.** Improvements could be made in the cleaning process, material and equipment selection, chemical selection and use as well as reducing reliance on traditional microbiological testing methods recognized as time consuming and laborious.

2.4.1 Current Legislative Requirements

In Australia, current legislative requirements dictate the use of microbiological testing in export and domestic meat processing establishments. The aim of this microbiological testing with respect to pre-operational hygiene is to verify the effectiveness of cleaning and sanitation programs. Key Performance Indicators (KPI) relating to product hygiene outcomes have been developed and consolidated into an index called the Product Hygiene Index (PHI). The KPIs included in the PHI have a direct bearing on product hygiene and/or the potential for product re-contamination.

AQIS require that in the system utilizing PHI, trends and their values are of significance rather than individual results. With respect to pre-operational hygiene, contact surfaces and personal hygiene are both used in compiling the monthly PHI score. The frequency of microbial counts in 5cfu/cm^2 ranges is noted and 'points' are deducted from the potential maximum total PHI score.

2.4.2 Potential Benefit of ATP

As results from microbial counts taken post cleaning and prior to production are not known until 48-72 hours later, there is the potential to use the immediate results from ATP bioluminescence measurement to assist in reduction of either microbial counts through additional cleaning or the removal of biofilms and other product residues.

While it is acknowledged that ATP provides rapid results for improved control of surface contamination and application of corrective action against poor hygiene, it is not a substitute for traditional culturing methods. However, a combination of ATP bioluminescence and traditional methods could provide many benefits if used as part of an overall integrated hygiene program.

Specific application of ATP bioluminescence in the Australian red meat industry could apply to areas of verification of the cleaning program, processes, identify abnormal cleaning events, and monitor trends, and initiate corrective actions such as re-cleaning. Further possibilities could be the use of ATP bioluminescence in training, offering real time identification of effective cleaning of personal protective equipment (PPE), equipment or other surfaces. Being able to immediately see the effectiveness of cleaning could result in an increased initiative and ownership from employees.

As a result of the simplicity of the ATP bioluminescence system it may also be possible to achieve cost savings from laboratory testing by optimising the amount of testing undertaken. Ultimately the use of an effective ATP hygiene system can help reduce product reject and recall levels, in turn protecting the consumer, the brand and retail relationships.

2.5 HOW ATP MACHINES WORK

A number of portable and easy to use ATP monitoring systems available today with many utilizing similar principles of operation. The main components comprising ATP bioluminescence hygiene systems include swabs, a luminometer (the ATP machine) and software (comes with the machine).

For ease of use, most swabs are 'All-in-One', containing the sterile swab usually pre-moistened, the liquid buffer and the enzyme and substrate (luciferase and luciferin) required to complete the reaction. In some brands, the reagents may be in a stable liquid or tablet form. Swabs are available for a diverse range of functions. The amalgamation of sample and reagents is normally achieved by reinsertion of the swab tip into the body of the swab and a twist or snap to release the reagent.

Light is generated when ATP is hydrolysed in a reaction that utilises the luciferin substrate and luciferase enzyme. Once the swab is mixed with the reagents it is then placed in the luminometer. This is device which measures light waves. ATP reacts with the luciferin-luciferase enzymatic complex and the light emitted is measured by the luminometer and expressed as Relative Light Units (RLU). The higher the amount of ATP on the surface, the higher the light output expressed as RLU.

It is important to note that the scale of readings of RLU are unique to each machine type and have no basis to any International Standards Organisation (ISO) measurement, making the nominal value of results specific to each model of device used. Manufacturers use individual scales of RLU to represent the amount of ATP. As the choice of scale is arbitrary, no advantage is conferred by systems having high or low response values.

Regardless of the RLU scale used, setting pass, caution and fail limits can be tailored to suit the surface type, location and risk associated. Most ATP hygiene systems come equipped with test-site specific programmable pass/caution/fail criteria.

Manufactures of these luminometers have made available functions for programmable testing regimes and individual testing site parameters, ensuring ease of use during routine testing. Data capture and manipulation can be a useful tool in Quality Management Systems. Data collected by the luminometer can be downloaded to a computer and manipulated using the relevant software. The data can be used to create customised reports, trend analysis graphs, identify problem areas, monitor retesting, and manage and track HACCP and SOP requirements. Use of good trending software can also be a useful aid during verification and audit.

2.6 REPEATABILITY, REPRODUCIBILITY AND SENSITIVITY

Understanding the value expressed by a luminometer as a part of an ATP hygiene system requires some knowledge of the variability which may be encountered. Just as all scientific measuring devices have differing levels of sensitivity and tolerance, so do luminometers. Repeatability and reproducibility are two of the more important features of performance luminometers.

Repeatability measures the success rate in successive experiments, possibly conducted by the same experimenters**,** test apparatus, and laboratory locations. The variation in the measurements is attributable to the system i.e. equipment and devices. Poor repeatability provides inconsistent results which can be difficult to interpret. Repeatability is often expressed as coefficient of variation (CV). The higher the CV, the more variation that can be expected in results from a given sample. As the CV increases, so do the number of false positives and false negatives. False positives may arise resulting in unnecessary re-cleaning of equipment. False negative results may put product and customers at risk.

Reproducibility refers to the ability of a test or experiment to be accurately reproduced, or replicated, by someone else working independently. Variation in the measurements is attributable to the user. Reproducibility is also measured by CV.

Good repeatability and reproducibility allows continuous incremental improvement in reducing threshold values of pass/caution/fail. It allows greater use and benefits from trend analysis of the data and informs the user about gradual loss of cleaning control or cleaning inconsistency.

Another important factor is sensitivity. Sensitivity as the measure of the smallest amount of ATP that can be detected by an ATP system and is a function of how much the test signal is greater than the background signal. Sensitivity determinations may be based on the lowest dilution capable of providing 5/5 positive replicate tests compared to the controls. Sensitivity is measured in fentomoles of ATP, and products on the market can vary from 0.5–2 fentomoles in sensitivity.

2.7 COMPARISON WITH MICROBIAL TESTING

Several research projects have been conducted to determine the ability of ATP-bioluminescence systems to detect and quantify microbial levels. While some studies have found a positive correlation under laboratory conditions, studies conducted under normal food processing conditions did not produce linear relationships with the number of microbes present on a surface. This is due to the presence of other materials containing ATP, such as food residues. ATP detected by this technique is derived from both microorganisms and somatic cells from plant and animals. Intracellular ATP is contained within living biological cells while extracellular ATP is located outside of biological cells that have been released from dead or stressed organisms. In addition, ATP concentration in microorganisms is

considerably higher in the logarithmic phase of bacterial growth than in the stationery phase. This means that while ATP bioluminescence testing may not necessarily quantify the levels of harmful microorganisms, detecting the presence of ATP indicates that a surface has not been thoroughly cleaned and may either harbour pathogenic organisms or provide a medium for their subsequent growth.

2.8 PRACTICAL LIMITATIONS OF ATP TESTING

There are limitations of ATP hygiene monitoring that need to be considered. The results of ATP hygiene monitoring will be affected by factors, such as pH and temperature that influence the reaction. The presence of detergents, sanitisers and other materials used in the sanitation process may diminish the amount of light produced from the bioluminescence process. These adverse effects may be overcome by the use of neutralising agents e.g. Tween 80 or lecithin.

ATP is generally a stable molecule as it withstands extraction by various methods e.g. acid, heat, organic solvents, detergents and sonic disintegration. ATP is highly soluble in water and is quite stable in solutions between pH 6.8–7.4, but is rapidly hydrolysed at extreme pH. ATP is an unstable molecule in unbuffered water.

There are reports that mineral oil as used in meat establishments may give false positive ATP readings. Food grade mineral oil or liquid petroleum is a liquid by-product of the distillation of petroleum. It is often used in establishments as a rust preventive on metal surfaces which have been cleaned and sanitised. Mineral oil contains no ATP thus will not give an ATP reading. However, certain brands may contain vegetable oils or have vegetable oils as a contaminant and will thus give an ATP reading. The same situation may occur on food grade grease. A validation test on the mineral oil used at an establishment would be advised as it may give false positive readings.

2.9 TRIALS DONE IN THE AUSTRALIAN MEAT PROCESSING INDUSTRY

An on-plant survey was conducted to ascertain if the measurement of ATP in meat processing establishments can be used in conjunction with microbiological testing to determine the state of preoperational hygiene.

The survey was conducted at two export beef processing establishments. The survey was repeated on the following day at each site, making a total of four sample collection days. Samples were collected from surfaces (n = 60) and personal protective equipment (PPE) (n = 42) at each site. The majority of the 120 food contact surface samples were from the slaughter floor and boning room (32 and 54, respectively) while the remainder were from various rooms such as offal room, gut/tripe rooms, packing areas and storage rooms. The PPE samples were taken from personnel prior to entry or prior to commencement of work in the slaughter floor and boning room (43 and 41, respectively). Three samples of different items of PPE were taken from each operator. Mostly operators were selected at random, and not necessarily chosen by the survey team or plant supervisors.

On the second day of the survey at one site, it was possible to repeat sampling of PPE for the same items on the same person, whose PPE had high ATP readings on Day 1, in order to undertake a case study on the operator's response to previous results. ATP and microbiological samples were taken by a trained and experienced microbiologist.

Two types of swabs were undertaken in parallel for each sample site, namely Surface Swabs using commercially available hygiene swabs; and Direct Contact plates using prehydrated 3M APC Petrifilm. This was due to there being no official sampling method for surface dictated by regulators. Day 1 involved using dry cotton tip surface swabs and hydrating the sample after each swab was taken. Day 2 involved using wet cotton tips and hydrating the sample before each swab was taken. These swab solutions were plated onto Petrifilm. A

comparison between dry and wet swabbing and direct contact Petrifilm methods was therefore able to be made.

Surface and personnel sampling and testing

Test method	*Day1, plant A & B*	*Day2, plant A & B*
ATP machine	✓	✓
Direct contact Petrifilm	✓	✓
Dry swab & Petrifilm	✓	
Wet swab & Petrifilm		✓

Each area was sampled by the respective swabs on an area of 100 cm^2. Every effort was made to take swabs side by side on the same surface areas. Each site was tested by ATP bioluminescence and Petrifilm aerobic plate count (APC) as per the table above.

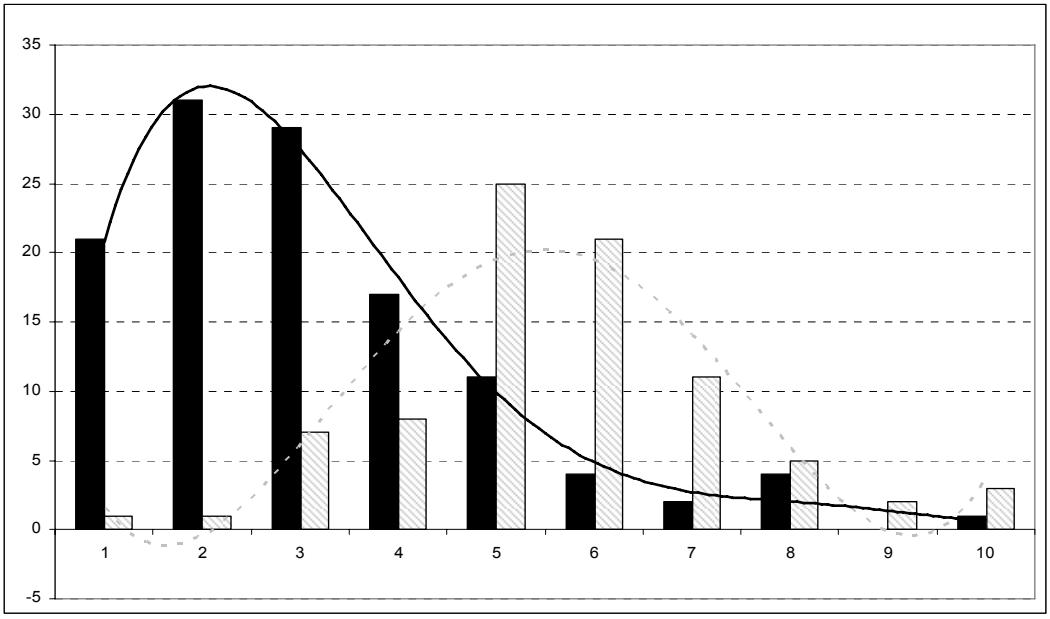
Sampling of contact surfaces was undertaken in parallel with the preoperational sampling carried out by the plant personnel. The sample sites for the survey were therefore selected at random using the establishment's random selection process. Where additional samples were required to be taken to meet the target sample numbers, these were selected by the project personnel from other sites from the establishment's random selection process that were not included in that production days' original selection. ATP machine results were expressed in RLU and APC in cfu/cm^2.

Summary of results and outcomes:

1. **The total results for personnel were much higher than for surfaces particularly for ATP RLU.** This may be explained by difficulty in cleaning and sanitising personal equipment e.g. mesh gloves, scabbards. The ATP machine would detect more animal cell ATP on personnel's equipment.

   Figure 1 indicates there is better hygiene of surfaces, albeit a skewed distribution, and a normal bell shaped curve for personnel. Surfaces show better cleaning but have long tail for a few very dirty surfaces. It may not be possible to achieve any cleaner to the left than 'normal' clean.

Figure 1. Frequency distribution of all surfaces (black columns) and all personnel (shaded) at plant A and B



2. **The correlation between the ATP RLU and APC results was very low.** This is due to the ATP machine detecting ATP in animal as well as bacteria cells whereas the APC only detects bacteria. Therefore by using ATP, there is scope to detect unclean surfaces which can harbour bacteria that are not detected using APC alone.

   Previous research has reported high correlations between log RLU and log APC counts (>0.9) on plastic and stainless steel surfaces which are devoid of animal and vegetable cells but seeded with bacterial cells. However, these reports acknowledge that the correlation in their studies is only valid for high counts of bacteria, such as >3 log. In the Australian survey, the predominance of low concentrations of bacteria and Not Detected results makes statistical correlations difficult unless there are huge sample numbers.

3. **Plant A has better ATP RLU results (lower levels) than Plant B for surfaces and personnel.** This indicates that the cleaning and sanitising procedures at Plant A were more effective than Plant B.

4. **There appeared to be 'project cleaning bias' on day 2 as both surface and personnel ATP RLU results were lower.** However, the significances for surfaces was lower (and not be considered strongly statistically significant). Interestingly, the personnel readings were more statistically significant suggesting there may have been a behavioural response to the hygiene monitoring.

5. **Plastic surface ATP RLU values were higher than stainless steel surfaces but the significance was not strong.**

6. **The ATP RLU results for surfaces were higher on the slaughter floor than the boning room.** However the significance was relatively weak.

7. **Direct contact Petrifilm was used as the 'gold standard' for comparison of dry and wet swabs used on Petrifilm.** There may be a slight advantage in using the direct contact Petrifilm or wet swab Petrifilm method for contact surfaces and the dry or wet swab Petrifilm method for personnel surfaces in order to obtain the highest APC count

8. **The targeted detection and prevention of high risk specific contaminated items could make a large difference to the hygiene of the plant.** The following surfaces made up 80% of the 120 samples for ATP RLU results: 2 wizz knives, 1 stainless steel belt, 1 kick plate and 1 plastic tub. For personnel gear the results were: 3 knives, 3 steels, 2 mesh gloves, 2 hooks and 1 pouch for the 84 samples.

   Thus a small proportion of samples made up the bulk of the scores. For example, elimination of high ATP RLU scores on these top 80% items would reduce the geometric mean of surfaces from 180 to 145 and personnel equipment from 2216 to 1372.

2.9.1 Setting ATP Limits from the Plant Survey

The setting of standards for a 'clean surface' are proposed to help manage the cleaning process. As noted, these standards must relate to specific instruments and swab combinations and are not transferrable between manufacturers. Therefore, the setting of Pass/Marginal/Fail parameters or other parameters for a particular machine, plant and/or items (eg food contact surfaces, personal equipment) is ideally undertaken for each establishment. Also, these parameters may change over time as further data is collected and the hygiene status changes.

After the purchase of an ATP machine it is important to develop Pass/Marginal/Fail parameters or other parameters. The pass/marginal/fail parameters for ATP measurements may be set statistically for more sophisticated users (see box below) or arbitrarily for simplicity.

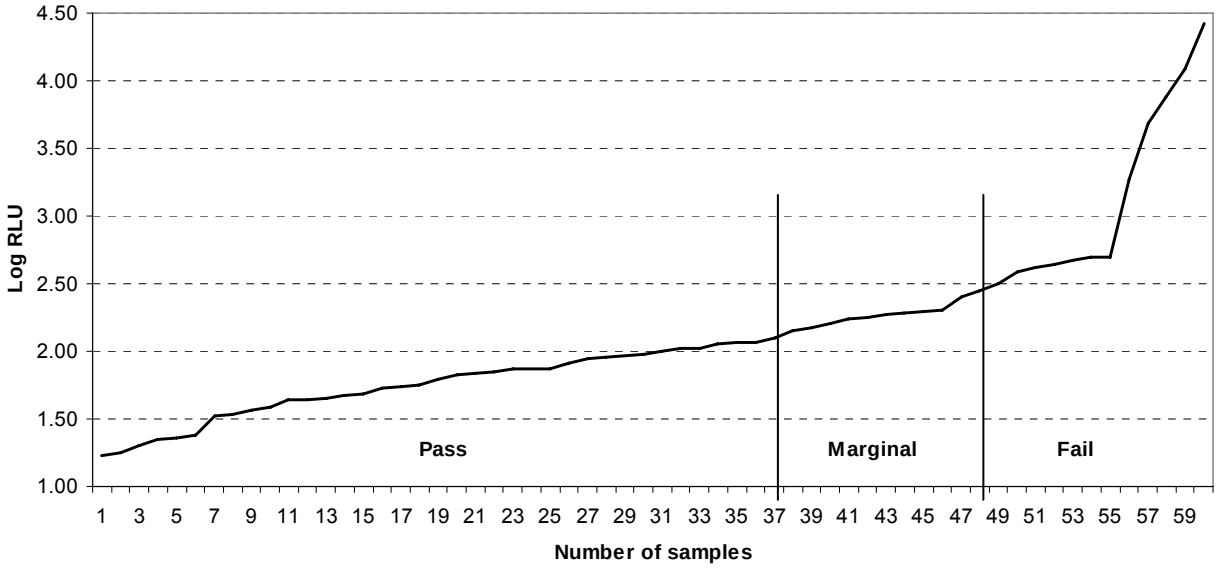
The Table below provides an example of different pass/marginal/fail parameters set statistically for each of the two establishments in this study. The same statistical methodology was used to develop the Pass/Marginal/Fail parameters for the ATP results. Plant B has higher RLU settings than Plant A for both surfaces and personnel. The separation of surface and personnel data is important due to the large difference in values.

Plant A			**Plant B**		
Surfaces (n = 60)	RLU	Ratio	Surfaces (n = 60)	RLU	Ratio
Pass	<133	62%	Pass	<244	52%
Marginal	133 – 284	18%	Marginal	244 – 612	20%
Fail	>284	20%	Fail	>612	28%
Personnel (n = 42)	RLU	Ratio	Personnel (n = 42)	RLU	Ratio
Pass	<1805	62%	Pass	<2722	50%
Marginal	1805 – 3654	19%	Marginal	2722 – 7367	24%
Fail	>3654	19%	Fail	>7367	26%

There are various methods of setting the parameters. It is important to reset the parameters over time. For example, if the hygiene is improving then resetting and lowering of the RLU will maintain approximately the same proportion in the Pass/Marginal/Fail brackets. This resetting may be weekly, monthly or longer.

Figure 2 shows an example of parameters developed from the survey data for surfaces at plant A (see table above). There would have been 37 (62%) Pass; 11 (18%) Marginal; and 12 (20%) Fail on 2 days. Of the 37 Pass, 6 samples were very good, and of the 12 Fail, 5 samples were very poor and warranted extra attention.

Figure 1 Parameters for surfaces at plant A



2.10 INCORPORATING ATP INTO PHI

The Product Hygiene Index (PHI) was introduced by AQIS as a means of verifying the on-going performance of export meat processing establishments. Establishments are required to submit their own data monthly to a central database. The PHI is a score out of a possible maximum of 100 each month, with reductions to that score (like points taken off) for results which fall outside the top one third of the pooled national results. Included in the calculation of PHI score are data for pre-operational hygiene results for personnel and surfaces.

The monthly PHI Data Submission sheet (MS Excel spreadsheet) requires meat processing establishments to present their pre-operational hygiene results for personnel and surfaces in bands of 5cfu/cm^2. For results in the bands of Not Detected and 0 – <5 cfu/cm^2 there is no reduction the PHI score for the month. All results above 5 cfu/cm^2 for both personnel and surfaces result in a reduction of 0.1 for every 1% of samples above this threshold. For example, if 15% of samples were >5 cfu/cm^2 for personnel and 8% of samples were >5 cfu/cm^2 for surfaces, this would result in a reduction of 1.5 and 0.8 to the monthly PHI score, or 2.3 in total.

AQIS has suggested the ATP results may be permitted to be used in lieu of micro test results for the PHI pre-operational hygiene results for personnel and surfaces. This is a possible alternative to establishing additional requirements in the PHI and changing the reporting Data Submission spreadsheets. An approach is to simply enter the number of ATP results that 'Fail' in place of the micro results that are above 5 cfu/cm^2. This alternative approach would require establishments to set their own 'Pass', 'Marginal' and 'Fail' limits. Given that this approach would be an alternative to limits set by AQIS, validation of the set limits would need to be undertaken and approved by AQIS before then being incorporated into the establishment's Approved Arrangement.

Another approach is to simply set up the ATP pass/marginal/fail limits, collect and analyse data and use the ATP measurements to drive improvements in the microbiological scores and assist in decreasing the reliance on microbial swabs only. This could result in a more targeted use of microbial testing and could still contribute to a higher PHI score through less fails for results >5cfu/cm^2.

At the time of submitting this report, AQIS has not provided any formal process for adoption of ATP measurement into the PHI. However, their support for the concept has been communicated verbally.

The outcomes of trials conducted in the Australian meat processing industry are:

- ***Detailed analyses of ATP and microbial test data showed that the two methods of determining pre-operational hygiene are complementary, not substitutes for each other.***
- ***Pass/fail/marginal limits could be set arbitrarily, however due to the non-normal distribution of surface ATP results these limits were better set by statistical means.***
- ***ATP measurements could be used to assist in maximising an establishment's PHI score.***

3. OPTIONS FOR UTILISING ATP MEASUREMENT

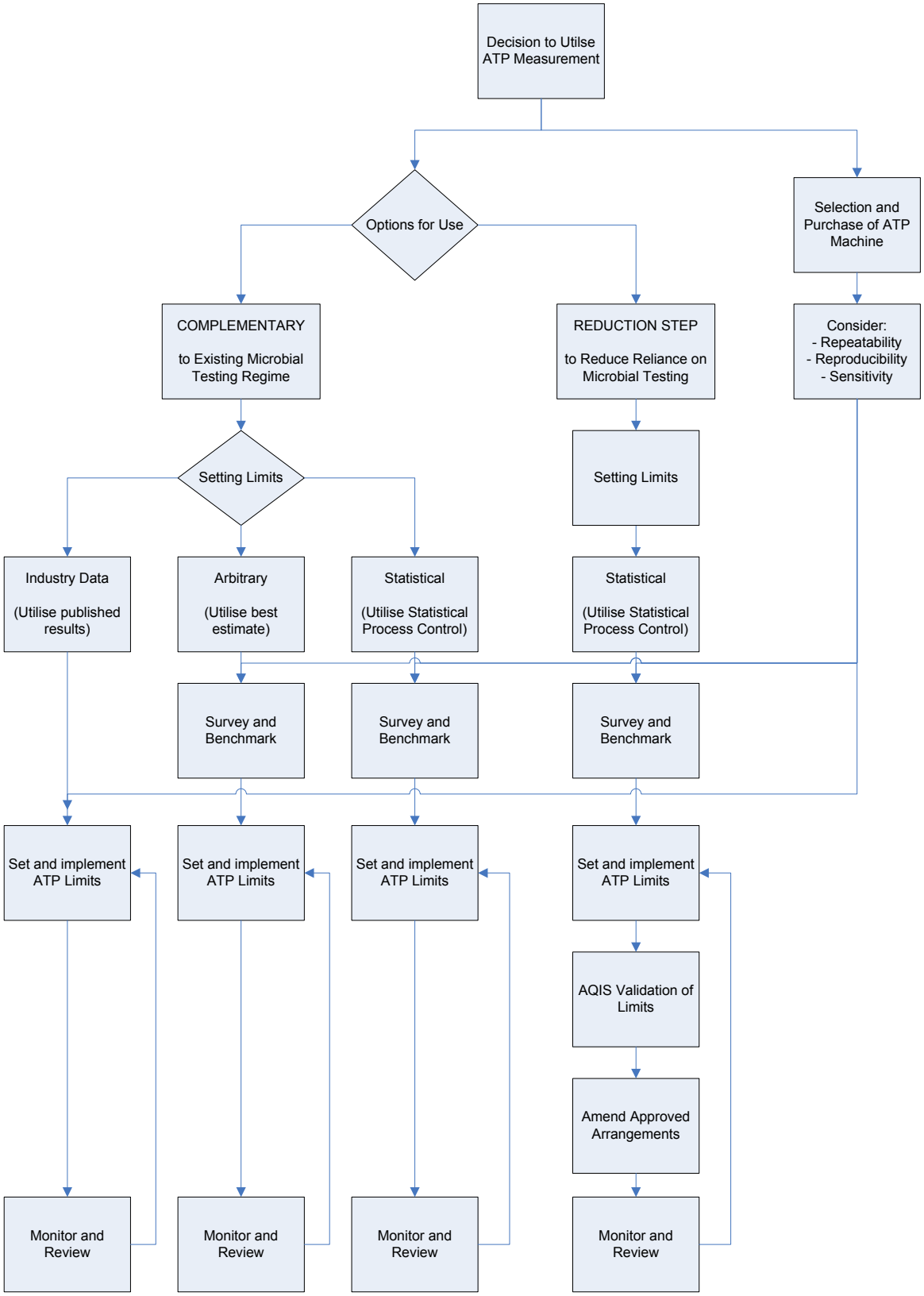
The use of ATP machines for pre-operational hygiene in other food processing industries such as dairy is well established. The general approach in those industries is to find and optimal mix of the real time ATP measurements and routine microbiological swabs.

Meat processing establishments have several options of how they can utilise and implement ATP measurement into pre-operational hygiene regimes in order to meet their specific needs and resourcing levels.

Figure 3 below provides a broad overview and decision making process for the options an establishment could follow when considering the integration of ATP measurement into their pre-operational hygiene testing procedures.

These options and the key points to consider for selection/purchase of an ATP Machine are further explained in the sections below.

Figure 3: Decision Tree for How to Incorporate ATP into Meta Processing Establishment's Preoperational Hygiene Monitoring



3.1.1 As a Complementary Measurement Tool Alongside their Microbial Testing

One option for meat processing establishments is to maintain their existing pre-operational hygiene regimes, and in addition, implement a regime of pre-operational ATP sampling, testing and reporting.

The benefits of this approach include:

- No requirement for alterations to their existing PHI reporting processes or establishment's Approved Arrangements
- Should still help PHI score by improving preoperational micro levels over time
- Establishments are able to set their own Pass/Fail/Marginal limits
- Validation of set limits (eg. Pass/Fail/Marginal) will not be required
- Setting and maintaining Pass/Fail/Marginal limits potentially less complex as not necessary to utilise statistical techniques.
- Instant objective feedback on the cleanliness of surfaces and equipment enabling immediate response and corrective action, facilitating proactive cleanliness management.
- Highly likely to drive behavioural change in personnel

The limitations of this approach include:

- No reduction in monitoring/verification costs
- The maintenance of parallel regimes
- No reduction to cost of microbial testing

In this option, establishments' Pass/Marginal/Fail limits for ATP measurements may be set utilising Statistical Process Control for more sophisticated users or set arbitrarily for simplicity. The limits can still be modified at any time.

3.1.2 As a 'Reduction Step' for Microbial Testing

Meat processing establishments have the option of incorporating ATP measurement into their existing pre-operational hygiene regimes to reduce the reliance on microbial testing.

The benefits of this approach include:

- Reduction of the amount and reliance on microbial testing, thus reducing the cost of microbial testing
- ATP results may replace APC results for PHI pre-operational Hygiene for personnel and surfaces
- Establishments are able to set their own Pass/Fail/Marginal limits
- Instant objective feedback on the cleanliness of surfaces and equipment enabling immediate response and corrective action, facilitating proactive cleanliness management.
- Potential reduction to monitoring/verification and audit costs

The limitations of this approach include:

- Requires revision to establishment's Approved Arrangement
- May submission to AQIS for review of validation process for ATP limits set
- PHI reporting parameters require revision
- Setting and maintaining Pass/Fail/Marginal limits more complex as it is necessary to utilise statistical techniques.

In this option, establishments' Pass/Marginal/Fail levels for ATP measurements must be set utilising Statistical Process Control.

3.1.3 Options for Setting Limits/Performance Criteria for ATP

Irrespective of the choice made above, establishments in Australia's meat processing industries have a number of options available in relation to setting ATP measurement limits and performance criteria. The choice of methodology will be dependant on the size, resourcing, customer requirements and attitude of each establishment to hygiene and continual improvement.

The setting of standards for a 'clean surface' are proposed to help manage the cleaning process and form part of pre-operational hygiene checks. **It is important to note that these standards must relate to specific instruments and swab combinations and are not transferrable between manufacturers of ATP measurement devices.** Therefore, the setting of Pass/Marginal/Fail parameters or other parameters for a particular machine, establishment and/or items (e.g. food contact surfaces, personal equipment) **needs to be undertaken for each establishment.** In addition, these parameters may change over time as further data is collected and the hygiene status changes.

Options that could be used to derive limits are as described below.

3.1.3.1 Other Industries

While it is useful to understand how ATP measurements are utilised in other industries eg. Dairy, the use of specific bio-luminescence levels for Pass/Fail/Marginal limits from other industries are not relevant to the meat processing industry. This is due to the different nature of surfaces, contact types, cleaning regimes and microbiological profiles. For these reasons, set limits are required to be established specifically for establishments within the meat processing industry.

3.1.3.2 Industry Data

An option available to meat processing establishments for setting Pass/Fail/Marginal limits is to use existing data from the Australian meat industry. At the time of publishing these guidelines, the only survey data available from the Australian meat processing industry can be sourced from the full report used to prepare these guidelines:

> *PROJECT P.PSH.0570*
> *"Selection and Use of ATP Machines for Hygiene Monitoring in Australian Meat Processing Plants"*
> *Prepared for Meat & Livestock Australia and 3M Food Safety*
> *December 2010*

This report summarises the survey Section 2.9 "TRIALS DONE IN THE AUSTRALIAN MEAT PROCESSING INDUSTRY" above.

This report is available from Meat and Livestock Australia, Sydney.

It should be noted that the results from this report relate specifically to the two processors surveyed. As the setting of standards relate to specific surfaces, measurement instruments, and swab combinations, the setting of Pass/Marginal/Fail parameters for a particular ATP machine is ideally undertaken for each combination at each establishment.

It is highly recommended if parameters are established based on industry data, that they be reviewed and revised over time as further data is collected at the specific establishment.

3.1.3.3 Company Data - Arbitrarily

The simplest method for establishing Pass/Fail/Marginal levels for ATP measurements is for the plant to arbitrarily choose the threshold levels. This process involves:

- Choosing an ATP measuring device to sample the relevant surfaces /equipment. Ensure adequate samples are recorded. Sampling regimes as used for Microbial testing may provide a suitable starting point.
- Establish relative target percentage ranges for Pass/Fail/ Marginal results e.g. 60% Pass, 20% Marginal, and 20% Fail.
- Rank all results for each subject and test (eg, Slaughter Floor/Boning room and Plastic/Stainless Steel).
- Identify the Relative Light Unit (RLU) levels that will provide the target proportions identified above eg. 60% Pass, 20% Marginal, and 20% Fail.
- Implement these levels and monitor the results in relation to Visual and Microbial assessments.

When utilising this arbitrary method to set ATP levels, it is recommended that the levels be monitored and revised relatively frequently when the system is initially implemented. It is important to monitor the results at regular intervals and reset the parameters over time. For example, if the hygiene is improving then resetting and lowering of the RLU will keep approximately the same proportion in the Pass/Marginal/Fail brackets. This resetting may be weekly, monthly or longer. This will drive an overall improvement in hygiene levels.

3.1.3.4 Company Data – Statistical

The most accurate methodology for establishing Pass/Fail/Marginal levels for ATP measurements are based on Statistical Process Control. Note that these levels must be validated by AQIS for inclusion into an establishment's Approved Arrangements if the establishment is proposing the ATP levels measurements be used as a reduction step in their hygiene regime.

An example of how to set limits statistically for a single establishment is outlined below.

The following is one example of how Pass/Fail/Marginal parameters may be set. The results in the table below are sourced from two plants surveyed in Australia and will be used for this example.

Plant A			**Plant B**		
Surfaces (n = 60)	RLU	Ratio	Surfaces (n = 60)	RLU	Ratio
Pass	<133	62%	Pass	<244	52%
Marginal	133 – 284	18%	Marginal	244 – 612	20%
Fail	>284	20%	Fail	>612	28%
Personnel (n = 42)	RLU	Ratio	Personnel (n = 42)	RLU	Ratio
Pass	<1805	62%	Pass	<2722	50%
Marginal	1805 – 3654	19%	Marginal	2722 – 7367	24%
Fail	>3654	19%	Fail	>7367	26%

Note: In practice the parameters would be rounded e.g. <133 could become <130; 133-284 could become 130-300; and >284 could become >300 and so forth.

1. It is preferable to divide the test results into distinct subjects eg surface and personnel equipment; slaughter floor and boning room; plastic and stainless steel, if applicable.

2. A minimum of 30 results should be used for each subject eg 30 contact surfaces.

3. It is best to record the information and results in a spread sheet or similar. Headings may include:
    - Date
    - Subject eg slaughter floor
    - Test eg surfaces
    - Results eg RLU and log10RLU.

4. All data is sorted on RLU or $\log_{10}$RLU in ascending order.

5. The mean and standard deviation (sd) of the $\log_{10}$RLU is calculated.

6. The sd is added to and subtracted from the mean to give outer limits.

7. The cut off for the Pass is the mean minus the sd as above plus this difference divided by 2.

8. This value is then converted back to whole numbers by taking the antilog of the value if $\log_{10}$RLU was used. This then becomes the cut off value for a Pass.

9. Similarly the Fail is the mean plus sd etc. This then becomes the cut off value for a Fail.

10. The Marginal value lies between Pass and fail points.

The Pass and Fail results also includes values lying outside the mean ± 1 sd. This information is important. For example, outside Fail values and their corresponding items need extra investigation. Possibly the item is poorly designed and can not be cleaned properly. Similarly and equally important, outside Pass values may indicate ideal and/or efficient cleaning and sanitising procedures, ideal surface etc. Alternatively, a smaller number of values outside the mean ± 2 sd can be investigated.

The method described is one example however, there are various methods of setting the threshold parameters statistically. However, is important to reset the parameters over time. For example, if the hygiene is improving then resetting and lowering of the RLU will keep approximately the same proportion in the Pass/Marginal/Fail brackets. This resetting may be weekly, monthly or longer. This will drive an overall improvement in hygiene levels.

3.2 SELECTION OF A SUITABLE ATP BIOLUMINESCENCE SYSTEM

There are many ATP systems on the market. The widespread adoption of ATP bioluminescence systems in food production facilities in North America and Europe has resulted in 2 major advances:

1. the instruments have decreased in size and price and increased in performance and utility
2. increased stability of the chemical reagents and ease of use of consumables.

Not all luminometers or swab reagents are the same. Improvement of instruments and reagents occur on a regular basis.

The following is a checklist which may be used to evaluate an ATP measuring system before purchase.

Table 1 Checklist for selection of an ATP bioluminescence system

	Research literature	***Manufacturer or distributor***	***Personal use***	***Other users***
Sensitivity	✓	✓		
Repeatability	✓	✓		

Reproducibility	✓	✓		
Ease of use			✓	✓
Size			✓	✓
Weight			✓	
Battery life		✓	✓	✓
Data software		✓	✓	✓
Download data		✓	✓	✓
Calibration		✓		
Guarantee		✓		
Backup service		✓		
Technical support		✓		
Purchase price		✓		
Cost per test		✓		

3.3 POINTS TO INVESTIGATE

ATP systems can vary considerably in their sensitivity, repeatability and reproducibility. A machine lacking sensitivity may fail to detect low levels of residual material, while a machine that lacks repeatability provides inconsistent results which may be difficult to interpret and act on.

Sensitivity, repeatability and reproducibility information is available in the literature or from the manufacturer or distributor. Laboratory trials are conducted under controlled conditions. These trials generally can not be conducted on-site. A consultant may be needed to interpret this information as it may be derived under different conditions and not be relevant to your situation.

On-site trials allow the ease and practicality of use to be assessed. For example, ease of swab activation, instrument battery life, size and weight of instrument, calibration of instrument, and robustness may be assessed. Additional information on instrument back up, reliability and technical support is best obtained from other users in conjunction with the manufacturers guarantees.

Good trend analysis software provided with the luminometer will make the capture, downloading and reporting of this information much easier.

The cost of the machine and cost per test can be obtained from the manufacturer or distributor.