Australian Government
Department of Health
Therapeutic Goods Administration

REQUEST FOR ADVICE

Advisory Committee on the Safety of Vaccines **Meeting 3**

25 October 2013

Item 3.1: Gardasil and premature ovarian failure

Product	Gardasil [Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant vaccine]
Sponsor	Merck Sharp & Dohme
Registered indication	Gardasil is indicated in females aged 9 through 45 years for the prevention of cervical, vulva, vaginal and anal cancer, precancerous or dysplastic lesions, genital warts, and infection caused by Human Papillomavirus (HPV) Types 6, 11, 16, and 18. Gardasil is indicated in males 9 through 26 years of age for the prevention of anal cancer, precancerous or dysplastic lesions, external genital lesions and infection caused by HPV types 6, 11, 16, and 18.
Summary of Issue/s	In July 2011, the TGA received a spontaneous report of a 16 year old girl who developed premature menopause following Gardasil vaccine (ADR 285383). The report was lodged by her GP (Dr s22 [redacted] which was published online in *BMJ Case Reports* on 3 October 2012. The article proposed an association between the vaccine and ovarian failure but did not postulate a biological mechanism for the association (Attachment 1). The TGA has been contacted by Dr s22 on a number of occasions about her view that there is a causal link between Gardasil vaccination and premature menopause/ovarian failure. The TGA has also responded to number of parliamentary questions on the issue. In 2013, Dr s22 has reported two cases of oligomenorrhoea in teenage females after Gardasil vaccine. The TGA has undertaken a safety filter for the issue of the association between Gardasil and premature menopause or ovarian failure (Attachment 2). The evaluators found 8 cases of premature menopause or ovarian failure in the WHO global database (Vigibase), including the Australian case, in the context of a global distribution of >97million doses

	of Gardasil and an expected occurrence of 10 cases of premature ovarian failure per100,000 person years in the 15-29 years age group. The evaluators concluded that there was no signal warranting further investigation. A recent article describing 3 cases of primary ovarian failure following HPV vaccination (vaccine unspecified) postulated that these cases were evidence of an autoimmune/inflammatory syndrome induced by adjuvants (ASIA) phenomenon associated with HPV vaccination (Attachment 3). Despite the authors' claim of increasing reports of post HPV-vaccine-linked autoimmunity, other authors have found no evidence of a link between other autoimmune conditions and HPV vaccine (Attachment 4).
Questions for ACSOV	1. Can the committee comment on whether there is any evidence of a biologically plausible explanation for premature ovarian failure following Gardasil vaccination?
Attachments	1. Little DT, Ward HRG Premature ovarian failure 3 years after menarche in a 16 years old girl following papillomavirus vaccination. *BMJ Case Reports* 2012. 2. Gardasil and Ovarian Failure – OPR safety filter 3. Colafrancesco S. Perricone C. Tomljenovic L. Shoenfield Y. Human papilloma virus vaccine and primary ovarian failure: another facet of the autoimmune/inflammatory syndrome induced by adjuvants. Am J Reprod Immunol 2013 Oct;70(4):309-16 doi:10.1111/aji.12151.Epub 2013 Jul 31 4. Macartney K, Chiu C, Georgousakis M, Brotherton J, Safety of human papillomavirus vaccines: a review. Drug Safety 2013: 36(4) online DOI 10.1007/s40264-013-0039-5.

Signed electronically *Date:* 11/10/13

Dr Jane Cook, Head, Office of Product Review

Delegate of the Secretary under regulation 39G(1)
of the Therapeutic Goods Regulations 1990

BMJ Case Reports

Findings that shed new light on the possible pathogenesis of a disease or an adverse effect

Premature ovarian failure 3 years after menarche in a 16-year-old girl following human papillomavirus vaccination

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Summary

Premature ovarian failure in a well adolescent is a rare event. Its occurrence raises important questions about causation, which may signal other systemic concerns. This patient presented with amenorrhoea after identifying a change from her regular cycle to irregular and scant periods following vaccinations against human papillomavirus. She declined the oral contraceptives initially prescribed for amenorrhoea. The diagnostic tasks were to determine the reason for her secondary amenorrhoea and then to investigate for possible causes of the premature ovarian failure identified. Although the cause is unknown in 90% of cases, the remaining chief identifiable causes of this condition were excluded. Premature ovarian failure was then notified as a possible adverse event following this vaccination. The young woman was counselled regarding preservation of bone density, reproductive implications and relevant follow-up. This event could hold potential implications for population health and prompts further inquiry.

BACKGROUND

Since secondary amenorrhoea and its causes may have great significance for a woman's future health, investigation of such presentations is warranted and is best addressed prior to potential masking by the oral contraceptives (OC). Subsequent diagnosis of premature ovarian failure, as in this young woman, will significantly affect her future health management. The occurrence of premature ovarian failure, previously known as premature menopause, in mid-teen years is extremely rare. The annual incidence of premature ovarian failure has been reported as 10/100 000 person-years between the ages of 15 and 29 years.[1] The cause of ovarian failure before age 40 years remains unknown in up to 90% of cases.[2] After diagnosis, evaluation for autoimmune disorder, genetic defect and exposure to ovarian toxins is important for counselling, surveillance for associated illnesses, for treatment and to further our understanding of the pattern of disease prevalence of premature ovarian insufficiency.

Recent data presented to the European Society of Human Reproduction and Embryology Conference in Stockholm in 2011[3] suggest that unexplained premature ovarian failure may have a current incidence sixfold greater than previously thought.

The unexplained occurrence of premature ovarian failure may reflect specific toxins or certain genotypes which currently available genetic examinations are not adequate to explore. Premature ovarian failure developing here, however, after human papillomavirus (HPV) vaccination prompted inquiry concerning ovarian histology of vaccinated rats at intervals of postvaccination. There was no record obtainable of these histological ovarian assessments. It also raised suggestions that long-term follow-up studies of natural cycles and fertility of vaccinated women should be considered.

CASE PRESENTATION

A 16-year-old girl presented with a history of 5 months amenorrhoea, preceded by approximately 12 months oligomenorrhoea. Menarche had occurred at the age of 13 in 2007 with initially light periods which became heavier and developed a regular monthly pattern over the following 12 months.

Early in 2009 menses became irregular. In early 2010 they became scant and occurred infrequently, two or more months apart. Menstrual periods ceased in January 2011. Following the development of amenorrhoea, the patient experienced hot flushes. She identified that an alteration in the menstrual pattern had started following HPV vaccination.

On first presentation to her local doctor she was prescribed the OC for amenorrhoea after exclusion of pregnancy. She elected not to take the contraceptive pill at that time and sought further opinion regarding her continuing amenorrhoea.

There was no past or present history of significant other illness, stressors or surgery, no known exposure to radiation or toxins and no other medications were being taken during or preceding this time. She was a non-smoker. There was no known family history of genetic abnormalities, premature menopause or of autoimmune disease. There were no abnormal findings on clinical examination; her weight was 56 kg, and body mass index was 22.6. The absence of a clinical basis for amenorrhoea prompted more evaluation.

INVESTIGATIONS

Further assessment revealed a normal full blood count, and normal renal, liver and thyroid function. Prolactin level and androgen profile were also within normal limits.

Follicle stimulating hormone (FSH) was raised at 108 U/l (menopausal range is 20–140 U/l); luteinising hormone was 31 U/l, (menopausal range is 10–65 U/l); estradiol was low at 63 pmol/l (normal follicular phase range is greater than 110, menopausal range is 40–200 pmol/l). Progesterone was low at 1.1 nmol/l (menopausal range is less than 2.2 nmol/l). Anti-Mullerian hormone was low at less than 1.0 pmol/l (levels below 14 pmol/l suggest failing ovulatory reserve). Serology was consistent with known previous mumps vaccination.

Karyotype was established as 46 XX. No ovarian antibodies were detected, and there were no adrenal antibodies. Thyroid peroxidase antibodies were 2 IU/ml and thyroglobulin antibodies were 44 IU/ml (levels up to 100 IU/ml can occur in normal subjects). Galactosaemia screen was negative (Gal-1-P uridyl transferase-RC was 0.42 U/g haemoglobin, the normal range is 0.26–0.52). Fragile X (Cytosine-Guanine-Guanine) n Repeats 28 was normal (normal range is less than 50). Pelvic ultrasound was normal.

DIFFERENTIAL DIAGNOSIS

The presence of menopausal gonadotrophin levels in association with over 3 months of amenorrhoea or oligomenorrhoea before age 40 years defines premature ovarian failure. Following an elevated FSH level it was next confirmed that this young woman's anti-Mullerian hormone demonstrated no measurable ovarian reserve. The exclusion of genetic causes such as Turner's syndrome, Fragile X and galactosaemia was necessary together with investigation for other endocrine or autoimmune disorders.

New South Wales Health has confirmed that three Quadrivalent Human Papillomavirus (types 6, 11, 16 and 18) Recombinant Vaccinations were administered to the client in the high-school vaccination programme in February, May and August 2008.

TREATMENT

This young woman was referred for specialist gynaecological review and management. She was advised of the need for adequate calcium, vitamin D, exercise and hormone replacement for bone density preservation. Implications for future childbearing and the need for periodic review were discussed. Hormone replacement was started in the form of the OC to treat menopausal symptoms as she approached matriculation studies. Plans were outlined for future follow-up of these issues together with monitoring side effects and complications of the contraceptive pill.

OUTCOME AND FOLLOW-UP

Baseline bone mineral density (BMD) was assessed, but standard references ranges for BMD do not extend to this patient's young age, so special reference ranges were used. These suggested femoral neck BMD of 0766 g/cm^2 to be in the low range for age, height and weight, and lumbar spine BMD of 0.903 g/cm^2 to be normal for height and weight but lower than the expected range for age. Interval reassessment is planned.

Premature ovarian failure has been notified as a possible adverse event to the Therapeutic Goods Administration of Australia (reference no. 285383) and to the company which produces this vaccine and to the sponsor.

Each 0.5 ml dose of the quadrivalent human papillomavirus virus-like particle vaccine (HPV VLP vaccine) contains proteins of HPV types 6, 11, 16 and 18; 225 mcg of aluminium (as amorphous aluminium hydroxyphosphate sulphate adjuvant); 9.56 mg of sodium chloride; 0.78 mg of L-histidine; 50 mcg of polysorbate 80; 35 mcg of borax and water.

It is not known whether this event of premature ovarian failure is linked to the quadrivalent HPV vaccine. More detailed information concerning rat ovarian histology and ongoing fecundity post-HPV vaccination was sought from the Therapeutic Goods Administration (TGA). Although the TGA's Australian Public Assessment Report for Human Papillomavirus Quadrivalent Vaccine, February 2011, does report on the histology of vaccinated rat testes and epididymides,[4] no histological report has been available for vaccinated rat ovaries.

The TGA subsequently agreed to a freedom of information application in the public interest (FOI 001-1112) requesting documented rat ovarian histology post-quadrivalent HPV vaccination that may have been performed by the sponsor and forwarded to the TGA. However, a histological report of the ovaries of vaccinated rats remained unavailable beyond a numbering of the corpora lutea present at postweaning euthanasia following the first litter (Extract Study no. TT#03-703-0(CTD Module 4, volumes 1–3) summary for non-clinical study report 'Intramuscular developmental toxicity and immunogenicity study in rats with postweaning evaluation').

DISCUSSION

Since there can be many causes of secondary amenorrhoea, from physiological to constitutional, systemic and failure of the hypothalamic–pituitary–ovarian axis, determining the aetiology requires broad considerations. In this woman it required exclusion of metabolic, other endocrine, autoimmune and genetic disorders.

Results consistent with premature ovarian insufficiency in a 16-year-old girl have significant consequences for her future health and for her prospects of motherhood.

Had this young woman taken the OC as prescribed for correction of her oligomenorrhoea/amenorrhoea, her diagnosis of premature ovarian insufficiency may not have been determined for perhaps some years. The possibility of its link to an adverse pharmaceutical event might also have been lost.

Anecdotal evidence from an informal discussion with high-school students suggests that one in three girls of this age is taking an OC for reasons of cycle control, acne management or for contraception. Given the prevalence of OC usage in this age group, combined with the possibility of initial OC prescription for the management of oligomenorrhoea (presumably to reduce associated anxiety, re-establish a 'normal' cycle and to protect bone mass, etc), conditions affecting menstrual function in this age group will be undetected and undiagnosed. Menstrual abnormalities and particularly ovarian insufficiency at this time may therefore be under-reported as possible adverse events following vaccination or other medication.

In addition, as the Australian sponsor of this vaccine has stated after notification: 'the postmarket reporting of adverse events is voluntary and from a population of uncertain size, and consequently it is not always possible to reliably estimate the frequency of these reactions or establish a causal relationship to product exposure'.

Learning points

- It is suggested that oligomenorrhoea and amenorrhoea even in young women be investigated prior to the start of the oral contraceptives.
- It is also suggested that development of oligomenorrhoea or amenorrhoea after establishment of regular menses be considered for notification as possible adverse events where they follow vaccination or medication.
- Assessment of vaccinated rat ovarian histology at intervals after vaccination is relevant and appropriate.
- Since there may potentially be a group for whom this vaccine is contraindicated, and since the occurrence of this event may possibly represent broader public health implications, it is also suggested that long-term follow-up of ovarian function in a cohort of vaccinated girls and women be undertaken.

Acknowledgements Helen Cleland Wyborn BSc (Nursing) (Chicago); Dip Med Ethics (Chicago); Michael Driscoll PhC; FACPP.

Competing interests None.

Patient consent Obtained.

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1. **Coulam CB,** Adamson SC, Annegers JF. Incidence of premature ovarian failure. *Obstet Gynecol* 1986;**67**:604–6.
2. **Nelson LM.** Primary ovarian insufficiency. *N Engl J Med* 2009;**360**:606–14.
3. **Islam R,** Cartwright R. Socioeconomic Class and Smoking Linked to Premature Menopause. Presented to European Society of Human Reproduction and Embryology Conference Stockholm 6 July 2011.
4. **Wise DL,** Jayanthi JW, Caplanski CV, *et al.* Lack of effects on fertility and developmental toxicity of a quadrivalent HPV vaccine in Sprague-Dawley rats. *Birth Defects Res (Part B)* 2008;**83**:561–72. Merck research laboratories, Westpoint, Pennsylvania.

SAFETY CONCERN FILTER	**Human papillomavirus vaccination and ovarian failure**	OPR Issue 4580 OPR Task 4452 TRIM record R13/764704

1. **TITLE: Human papillomavirus vaccination and ovarian failure**

2. **WHAT IS THE SOURCE OF THE SAFETY CONCERN?**

In July 2011, the TGA received a spontaneous report of a 16 year old girl who developed premature menopause following Gardasil vaccine (ADR 285383). The report was lodged by her GP (Dr s22 [redacted] s22 [redacted] s22 [redacted] which was published online in *BMJ Case Reports* on 3 October 2012[1].

The TGA has been contacted by Dr s22 [redacted] on a number of occasions about her view that there is a causal link between Gardasil vaccination and premature menopause/ovarian failure. The TGA has also responded to number of parliamentary questions on the issue.

3. **WHAT PRODUCTS ARE INVOLVED?**

- The product is Quadrivalent Human Papillomavirus (Types 6,11,16,18) Recombinant Vaccine (Gardasil).
- Does the safety issue pertain to specific conditions of use (e.g. dose, route)? No. Gardasil is a sterile preparation for intramuscular administration.

4. **WHAT IS THE SAFETY CONCERN?**

The safety concern is whether there is a possible causal association between Gardasil vaccine and premature menopause/ovarian failure as postulated by the authors (Little and Ward) of the case report in *BMJ Case Reports.*

The case report describes a female who received 3 doses of Gardasil in the 4 months before her 14th birthday (February, May and August 2008). Menarche had been at age 13 years with establishment of a regular monthly pattern over the following 12 months. Early in 2009 her periods became irregular and from early 2010 they became scant and infrequent until she ceased to menstruate in January 2011. Investigations confirmed premature ovarian failure. Other investigations excluded possible genetic causes and a number of possible underlying metabolic, endocrine and autoimmune disorders.

The authors have not proposed a biological mechanism for a causal association between Gardasil and premature ovarian failure.

The authors raised concern that the current practice of using oral contraception to regularise menstrual periods in young women may be masking other potential cases related to vaccination or other medication. One of the authors' recommendations is that long-term follow-up of ovarian function in a cohort of vaccinated girls be undertaken,

- *Does the concern apply to sub-groups or to the whole target population?*
  This concern applies to females under 40 years of age (the age before which ovarian failure is considered premature) who have received Gardasil vaccine.

- Estimate the severity or range of severities of the AE/ADR (if possible):
  The AE described in the single case report is classified as severe.

- Estimate the likelihood of the AE/ADR (if possible):
  The incidence of premature menopause in the age group 15 to 29 years has been estimated at 10/100,000 person years.[2]

5. HAS THE ISSUE BEEN DEALT WITH PREVIOUSLY?

No. This is the only case reported to the TGA. The non-clinical data evaluated prior to registration did not show any effect on fertility in female rats.

- *Does the current Product Information PI (if one exists) fully capture this issue?*
  The PI does not mention premature menopause or ovarian failure as adverse events.

- *If the PI refers to this issue, does the new safety signal suggest any significant change to the magnitude or scope of risk, or certainty of causality?*
  Not applicable - a signal has not been detected.

- *Has this (or an overlapping) issue already been considered by the TGA?* NO☐

6. IS THE SAFETY CONCERN POTENTIALLY VALID?

- *Can the source of the safety concern be dismissed as unreliable / inaccurate?*
  The validity of the issue is uncertain. While the case report has been published, it is a single report and no biological mechanism for the association has been postulated.

7. IS THE SAFETY CONCERN POTENTIALLY RELEVANT? YES☐

- *Are the product/s on the ARTG?* Y☐
  - Are there implications for related products that are on the ARTG? Y☐
    Bivalent HPV vaccine (Cervarix)
  - *How widely used are the product/s?* Gardasil has been offered to all 13 year old females in school vaccination programs since 2007, with follow-up doses offered in general practice and council health clinics in some jurisdictions. . A catch-up program for females aged 14 to 26 years was also offered between April 2007 and December 2009 in school-based programs (14-18 years) and in general practice and other clinics (18-26 years). To date, in Australia around seven million doses have been distributed. HPV vaccination is administered in 120 countries worldwide with over 97 million doses being distributed.
  - Indication/s: Prophylactic protection against human papillomaviruses which can result in genital warts, dysplasias and cancers of the cervix, vulva, vagina, penis, anus and the oropharyngeal cavity.

- Schedule: HPV vaccination is included on the National Immunisation Program as a 3-dose schedule. From the beginning of 2013 it is being offered to females and males aged 12-13 years in schools with a 2 year catch-up program for boys aged 15-17 years.
- PBS? NO ☐ RPBS? NO ☐

- Briefly summarise the ADR Database picture of this issue:

  - As at 3 October 2013, the ADRS recorded a total of 2725 case reports for suspected AEFI with Gardasil vaccine, HPV quadrivalent vaccine and HPV vaccine not otherwise specified.
  - Of these, there was one report of premature menopause or premature ovarian failure (case 285383 described above).
  - To explore the possibility of unrecognised ovarian failure, the case reports were searched for cases of amenorrhoea or oligomenorrhoea which had not recovered at the time of reporting. This search identified seven relevant case reports (234473, 235230, 239273, 256945, 313165, 313166 and 326037). Two of these cases (313165 and 313166) were reported to the TGA by Dr s22
  - A summary of the review of each case report is provided below:
    - Case 234473 is a 17 year old female who received Gardasil dose1 on s22. Her last menstrual period occurred during the third week of s22 with no period since. At the date the report was made (s22) she continued to have amenorrhoea. No follow up report has been received and long term outcome is unknown.
    - Case 235230 is a 16 year old female who received Gardasil dose 2 on s22 at which time she reported that she had not menstruated for the two months since commencing the HPV program. Prior to this, menses had been regular "since age 16 years". The TGA subsequently received information that the parents could not be contacted for follow-up and the long-term outcome is unknown.
    - Case 239273 is a 25 year old female who had delayed cycles post Gardasil doses 1&2. She developed amenorrhoea after dose 3 (given on s22) which persisted at the time of the report in s22. No follow up is available and the long-term outcome is unknown.
    - Case 256945 is a 16 year old female who received Gardasil dose 2 on s22 after which she experienced general muscle weakness, headaches, fatigue and sweating. She was subsequently hospitalised for a week and diagnosed as having post viral fatigue by an immunologist. These symptoms were still present at the time of reporting in s22. The report also states that she "did not menstruate between dose one and dose two" of the HPV vaccine. There is no information about whether the amenorrhoea continued following dose 2.

- Cases 313165 is a report made in s22 [redacted] of a 15 year old female who received her first dose of Gardasil in 2011, at the end of year 8, with a second dose given in 2012 (exact dates unknown). After her first dose her periods changed from being heavy to being light and irregular. When last seen (date unknown) she had continuing scant bleeding and a long cycle.
- Case 313166 is a report made in s22 [redacted] of a 16 year old female who received Gardasil vaccine in Years 8 and Year 9. Menarche occurred subsequently in (?) February 2011 "at the age of 15 years". Periods became irregular from mid 2011 and she had two to four periods a year during 2011-12. In 2013 her periods became more regular. There is a family history of polycystic ovary syndrome (PCOS) but investigations ("pelvic US and bloods") indicated that she did not meet the Rotterdam criteria for PCOS. She has been advised to increase her weight to BMI >20.
- Case 326037 is a report made in s22 [redacted] of a 13 year old female who received Gardasil dose 1 with HBVax on s22 [redacted] and Gardasil dose 2 with Boostrix on s22 [redacted]. Immediately after dose 2 she had a syncopal seizure, headache and blurred vision. She had had regular menses since menarche at age 9 years but only 1 light period since dose 2 of Gardasil.

- There were an additional 17 case reports for Cervarix in the ADRS database but no reports of premature menopause, ovarian failure, amenorrhoea or oligomenorrhoea.

- The WHO database, VigiBase, was searched on 9 October 2013. There were two cases of ovarian failure, six cases of premature menopause (one of which is the Australian case) and 130 cases of oligomenorrhoea and/or amenorrhoea following Gardasil vaccine in females under 40 years. All cases other than the Australian case were reported from the USA. The age range of the cases was 13 to 23 years. Ulcerative colitis was a concomitant condition in one case of ovarian failure and one of the US cases of premature menopause also reported an "autoimmune disorder".

- A literature search was undertaken in July 2013 with the assistance of the TGA Library to identify published medical literature on premature menopause or ovarian failure following HPV vaccination. Databases searched included PubMed and Embase. The contents pages of online journals were also searched. No information relating to ovarian failure and HPV vaccination apart from the *BMJ Case Reports* article was identified. A further search in PubMed and Embase on 10 October 2013 identified an article by Colafrancesco et al published in the October 2013 issue of the American Journal of Reproductive Immunology which identified three cases of young women who developed secondary amenorrhoea following HPV vaccination[3]. All three were subsequently diagnosed as primary ovarian failure. In two cases specific auto-antibodies were detected (anti-ovarian and

anti-thyroid) which the authors state as "suggesting that the HPV vaccine triggered an auto-immune response". The authors suggested that these cases are evidence of an autoimmine/inflammatory syndrome induced by adjuvants (ASIA) phenomenon related to HPV vaccine

8. RECOMMENDATIONS:

- Review is required NO ☐
  - Reason/s for **not** requiring a review:

This filter has not identified a safety signal. There are 8 reports of premature menopause or ovarian failure globally, including the Australian case. Premature ovarian failure has been estimated to occur in the 15-29 year old age group at a rate of 10 per 100,000 and is found to be idiopathic in up to 90% of cases. Given the large number of Gardasil doses administered in Australia (>7 million) and globally (>97 million), it would be anticipated that cases of premature menopause or premature ovarian failure would occur as a chance occurrence after Gardasil vaccination. The number of case reports is very small and does not suggest an occurrence greater than would occur by chance.

The Colafrancesco et al article has proposed an ASIA phenomenon to explain the association between HPV vaccination and ovarian failure. However, ASIA appears to be a hypothesis largely propounded by Dr Y Shoenfield, who is a co-author on the article, to explain a range of autoimmune conditions. Despite the claims of the authors, there is little evidence of an association between HPV vaccination in general, and Gardasil vaccination in particular, and the occurrence of a range of autoimmune conditions other than would be expected by chance.[4]

Oligomenorrhoea and amenorrhoea in adolescence can relate to a number of factors, such as establishing regular menstruation, weight fluctuation and stress and is a common occurrence, especially in the first 2-3 years following menarche. The ADRS and Vigibase case reports of oligomenorrhoea and amenorrhoea provide insufficient information for evaluation and a lack of long term follow up. The number of reports is relatively small given the number of doses distributed and the frequency of these conditions in the age group receiving HPV vaccine.

- Any further reports of premature menopause, amenorrhoea or oligomenorrhoea following HPV vaccine should be reviewed by a Medical Officer as they are entered into the ADRS.

9. REFERENCES:

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Evaluator:

Name: s22 [redacted] Date: 31 July 2013

Updated by s22 [redacted] 11 October 2013

Authoriser comments:

Authoriser:

Name: Jane Cook Date: 24 September 2013

Reauthorised (updated filter)

Name: Jane Cook Date: 11 October 2013

ORIGINAL ARTICLE

Human Papilloma Virus Vaccine and Primary Ovarian Failure: Another Facet of the Autoimmune/Inflammatory Syndrome Induced by Adjuvants

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Keywords
Autoantibodies, autoimmune/inflammatory syndrome induced by adjuvants, autoimmunity, human papilloma virus, primary ovarian failure

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Submission April 24, 2013;
accepted June 25, 2013.

Citation
Colafrancesco S, Perricone C, Tomljenovic L, Shoenfeld Y. Human papilloma virus vaccine and primary ovarian failure: another facet of the autoimmune/inflammatory syndrome induced by adjuvants. Am J Reprod Immunol 2013; 70: 309–316

doi:10.1111/aji.12151

Problem
Post-vaccination autoimmune phenomena are a major facet of the autoimmune/inflammatory syndrome induced by adjuvants (ASIA) and different vaccines, including HPV, have been identified as possible causes.

Method of study
The medical history of three young women who presented with secondary amenorrhea following HPV vaccination was collected. Data regarding type of vaccine, number of vaccination, personal, clinical and serological features, as well as response to treatments were analyzed.

Results
All three patients developed secondary amenorrhea following HPV vaccinations, which did not resolve upon treatment with hormone replacement therapies. In all three cases sexual development was normal and genetic screen revealed no pertinent abnormalities (i.e., Turner's syndrome, Fragile X test were all negative). Serological evaluations showed low levels of estradiol and increased FSH and LH and in two cases, specific auto-antibodies were detected (antiovarian and anti thyroid), suggesting that the HPV vaccine triggered an autoimmune response. Pelvic ultrasound did not reveal any abnormalities in any of the three cases. All three patients experienced a range of common non-specific post-vaccine symptoms including nausea, headache, sleep disturbances, arthralgia and a range of cognitive and psychiatric disturbances. According to these clinical features, a diagnosis of primary ovarian failure (POF) was determined which also fulfilled the required criteria for the ASIA syndrome.

Conclusion
We documented here the evidence of the potential of the HPV vaccine to trigger a life-disabling autoimmune condition. The increasing number of similar reports of post HPV vaccine-linked autoimmunity and the uncertainty of long-term clinical benefits of HPV vaccination are a matter of public health that warrants further rigorous inquiry.

Introduction

Vaccines against human papilloma virus (HPV) are thought to represent a useful approach in the fight against cervical cancer. Although vaccines have proven to be a successful and cost-effective asset for preventive medicine, local or systemic adverse events, following vaccination, have been described. Specifically, there are increasing reports that autoimmune disorders can develop after vaccination.[1 4] At the same extent, the association between infectious agents exposure and the development of autoimmune diseases is well established.[5,6] Recently, a new syndrome, namely the autoimmune/inflammatory syndrome induced by adjuvants (ASIA) or Shoenfeld's syndrome,[7 12] has been defined, alluding to the key role of adjuvants in inducing autoimmunity. The syndromes included in ASIA entail immune-mediated conditions that appear following a chronic stimulation of the immune system by agents with adjuvant characteristics.[7,10] Post-vaccination autoimmune phenomena represent a major issue of ASIA and different vaccines, including the HPV vaccine, have been found as possible causes.[3,9,13] Primary ovarian failure (POF) is a clinical condition with complex aetiology in which autoimmune mechanisms represent 20–30% of the cases.[14] This assertion is supported by different evidences: the presence of lymphocytic oophoritis, the detection of ovarian autoantibodies and the frequent association with other autoimmune diseases.[14] Herein, we describe three clinical cases, including two sisters, who developed POF following administration of the HPV vaccine. Genetic, metabolic and external environmental factors were excluded as POF causes, while the common denominator was the previous vaccination with HPV leading to the development of immune-mediated amenorrhoea.

Case 1

A young previously healthy girl received three administrations of the quadrivalent HPV vaccine (T0, T1 after 4 months, T2 after 9 months) when she was 14 years old. Six months before the first injection, the patient had menarche. Her psycho-physical and sexual development were normal except that at the time she received the first HPV vaccine dose, she was complaining of irregular periods (every 2 months). After the first vaccination, the patient immediately started to complain of burning and heavy sensation in the injected arm, followed by skin rash and fever. Nausea and stomach aches lasted for 2 days after the injection, while in the subsequent 2 weeks, she further complained of cramping and headache. At the time of the second vaccine administration, she reported similar injection site related symptoms, accompanied by sleep disturbances, such as insomnia and night sweats. At the time of the third injection, the patient continued to experience the same symptoms: burning, pain and heavy sensation in the injected arm, headache and cramping. Insomnia associated with night sweats persisted and she started complaining of arthralgia, anxiety and depression. The patient reported that her last period occurred shortly after the last injection of the HPV vaccine. The hormonal screening showed the presence of increased follicle-stimulating hormone (FSH) and luteinizing hormone (LH) associated with very low levels of estradiol. Beta human chorionic gonadotropin (HCG) tested negative excluding pregnancy. The karyotype study was 46 XX, while molecular studies ruled out Fragile X syndrome and mutated follicle-stimulating hormone receptor (FSHR) gene. A pelvic ultrasound did not show any abnormality. According to these clinical and serological findings, POF diagnosis was determined. Even though the patient started therapy with medroxyprogesterone to stimulate bleeding, no improvement occurred and she continued to experience abnormal vaginal bleeding, night sweats, hot flashes and sleep disturbances.

Case 2

This patient (the younger sister of the above-mentioned case) received three administrations of the quadrivalent HPV vaccine at the age of 13 under the same protocol as her sister. At that time, she had normal growth and sexual development. The patient complained, 10 days after the first injection, of general symptoms such as depression and sleep disturbances. She also experienced episodes of lightheadedness and tremulousness, anxiety, panic attacks and difficulties in focusing/concentrating in her school work. She had menarche at the age of 15 years, followed by another period 1 month later and none thereafter. Laboratory analysis showed high serum levels of FSH and LH with undetectable estradiol. The genetic test for Turner's syndrome,

Fragile X syndrome and FSHR gene was performed and resulted negative. Interestingly, the patient tested positive for antiovarian antibodies. She underwent a pelvic ultrasound without an evidence of abnormalities. In the light of these findings, a diagnosis of POF was determined and the patient was treated with several different hormonal replacement therapies with a poor therapeutic response.

Case 3

The patient received the quadrivalent HPV vaccine in three administrations (T0, T1 after 2 months, T2 after 4 months) at the age of 21 years. Menarche occurred when she was 13 years old with normal monthly periods and a flow of 5–7 days, with mild cramps. A normal sexual development was reported. Few months after the last injection of HPV vaccine, she started complaining of irregular menses (off by 1–2 weeks) without an increase in bleeding or pain. The irregular periods worsened and the patient reported on menstruations every 3 months with bleeding only for 2 days. For this reason, she started drospirenone/ethinyl estradiol. Nonetheless, no improvement occurred and after discontinuation of therapy, at the age of 23 years, she complained of amenorrhoea. The laboratory tests showed the presence of very low levels of estradiol and increased FSH and LH. Testosterone, cortisol and prolactin serum level were found normal. Although the thyroid hormones were also in the normal range, the patients had positive antithyroid peroxidise (TPO) antibodies (134 IU/mL, n.v. 0–34). The karyotype evaluation and the search for Fragile X syndrome displayed no aberrations. A transvaginal and pelvic ultrasound did not reveal any abnormality. According to these findings and clinical features, a diagnosis of POF was determined. Thus, a therapy with medroxyprogesterone and estradiol was attempted, however, it did not improve her clinical condition.

Discussion

Herein, we have described three cases of POF following HPV vaccination. To the best of our knowledge, an additional case of POF in a 16-year-old young woman who was vaccinated with the quadrivalent HPV recombinant vaccine has already been reported by Little and Ward.[15] In this case, as in our three cases, no other possible causes of POF were identified other than the HPV vaccine. Quoting the HPV vaccine manufacturer, the authors emphasized the fact that the post-marketing reporting of vaccine adverse events is voluntary and consequently, it is not always possible to reliably estimate the frequency of such reactions, let alone to establish a causal relationship to the vaccine. Further according to the authors, there may potentially be a group for whom the HPV vaccine is contraindicated and because the occurrence of POF carries major health implications, a long-term follow-up of ovarian function in a cohort of HPV vaccinated woman should be undertaken.[15]

POF is a syndrome consisting of primary or secondary amenorrhoea, hypergonadotropinemia and hypoestrogenemia. POF affects 1% of women under 40 years of age, 0.1% under 30 and 0.01% of women under 20 years and it is an important cause of infertility and psychological stress.[14] POF in young women can indeed have significant consequences for future health and prospects of motherhood. The aetiology includes specific genetic mutations (referred to oocyte, enzymes or hormones receptors), autoimmune or environmental causes (such as viral infections, chemotherapy, radiotherapy and pelvic surgery) or metabolic disturbances.[14] The possible autoimmune origin for POF has been speculated for a long time,[16] and one of the evidence which supports this origin is its frequent association with other autoimmune diseases (i.e. thyroiditis, Addison's disease, autoimmune polyglandular syndrome, systemic lupus erythematosus, Sjogren's syndrome, haemolytic anaemia and idiopathic thrombocytopenic purpura).[17] The presence of autoantibodies reactive to different parts of the ovary has been detected in many POF cases and the most commonly recognized autoantigens are on the ooplasm, theca, granulose, corpus luteum or zona pellucida.[18 20] More specific antigenic targets of autoantibodies have been identified in steroid cell enzymes including 3b-hydroxysteroid dehydrogenase (3b-HSD), cytochrome P450 side-chain cleavage enzyme (P450SCC) and 17α-hydroxylase/17,20 lyase enzyme (CYP17A1).[14] Nonetheless, the detection of such antibodies has yielded conflicting results because of the different stages of disease in which the tests were conducted, methodological differences and the multiplicity of potential immune targets. In our cases, only one of the three patients had positive antiovarian antibodies. Given the difficulties in detecting these antibodies, an autoimmune origin of POF may be speculated for the other two cases. Indeed, the pres-

Table I The Suggested Criteria of Autoimmune/Inflammatory Syndrome Induced by Adjuvants (ASIA)[7] in the Current Three Cases of Post Human Papilloma Virus Vaccine Manifested Primary Ovarian Failure (POF). Note That for Positive Diagnosis of ASIA, Fulfilment of Either Two Major or One Major and Two Minor Criteria is Required

	Case 1	Case 2	Case 3
Major criteria			
1. Exposure to an external stimuli (infection, vaccine and/or immune adjuvants) prior to clinical manifestations	+	+	+
2. The appearance of 'typical' clinical manifestations;			
Myalgia, muscle weakness	–	–	Not reported
Arthralgia and/joint pain	+	–	–
Chronic fatigue, un-refreshing sleep or sleep disturbances	+	+	Not reported
Neurological manifestations	+	+	Not reported
Cognitive disturbances	–	+	Not reported
Pyrexia	–	–	–
3. Removal of inciting agent induces improvement	NA	NA	NA
4. Typical biopsy of involved organs	Not assessed	Not assessed	Not assessed
Minor criteria			
1. The appearance of autoantibodies (antiovarian, anti-TPO)	–	+	+
2. Other clinical manifestations (e.g. amenorrhoea)	+	+	+
3. Specific HLA (e.g. HLA DRB1, HLA DQB1)	Not assessed	Not assessed	Not assessed
4. Evolvement of an autoimmune disease (POF)	+	+	+

ence of antiovarian antibodies in the second case, in addition to the finding of the anti-TPO antibodies in the third case, lends support to the idea that autoimmune responses underlying POF can develop following HPV vaccination. Moreover, as POF developed in two sisters, a genetic susceptibility predisposing to post-vaccination POF is probable. The very unusual early age of disease onset may reinforce this suggestion as it was already observed in other immune-mediated diseases.[21,22] Furthermore, the patients experienced not only POF but also a constellation of other symptoms, including arthralgia, sleep disturbances and cognitive dysfunction, consistent with the diagnosis of the ASIA syndrome (Table I).[7,9]

POF as a Part of the ASIA Syndrome

The three cases of POF described herein clearly fulfilled the criteria for the ASIA syndrome (Table I). ASIA comprises a group of diseases including post-vaccination phenomena,[9,11,13] silicone implant–induced autoimmunity,[23] Gulf War syndrome,[24] macrophagic myofasciitis with chronic fatigue syndrome[25,26] and the sick-building syndrome[27] which share a common set of signs and symptoms. Shoenfeld and Agmon-Levin[7] proposed four major and four minor criteria for ASIA (Table I), and to diagnose ASIA, fulfilment of either two major or one major and two minor criteria is required. The criteria for ASIA enable the inclusion of patients with well-defined autoimmune diseases (i.e. multiple sclerosis, lupus) as well as those with ill-defined and non-specific yet clinically relevant conditions (i.e. myalgia, chronic fatigue and cognitive disturbances) under the spectrum of vaccine adjuvant-associated conditions.[9] The inclusion of the latter category of manifestations under ASIA is of special importance as these non-specific manifestations are all too easily ignored or disregarded as irrelevant and non-vaccine related not only by patients and physicians, but also by scientists involved in design of vaccine trials.[28,29] Nonetheless, many ill-defined medical conditions that fall under the ASIA spectrum are frequently disabling and thus of significant clinical relevance.[9,25]

Apart from a shared set of clinical manifestations, the other main common feature in ASIA is the presence of an immune adjuvant. An adjuvant is defined as 'any substance that acts to accelerate, prolong or enhance antigen-specific immune response'.[24] The adjuvant is able to stimulate the immune system and to increase the response to a vaccine, without having any specific antigenic effect in itself.[24] Vaccines, which contain infectious antigens either attenuated or recombinant, may induce autoimmunity by means of similar 'infectious' mechanisms such as molecular

mimicry, epitope spreading, bystander activation and polyclonal activation.[30,31] When this occurs, it can be subacute or sometimes a long time after the vaccination (i.e. months to years),[32 37] which leads to difficulties in identifying a definite causality between vaccination and autoimmune phenomena. The latter will most commonly occur in genetically predisposed individuals. Indeed, personal or familial susceptibility to autoimmunity and adverse response to a prior dose of the vaccine both appear to be associated with a higher risk of post-vaccination autoimmunity.[3,9]

HPV Vaccines and Autoimmunity

In the current literature, there are numerous cases substantiating the link between adverse immune reactions and HPV vaccines, including fatal reactions. For example, Lee[38] recently reported a case of a teenage girl who underwent sudden unexpected death approximately 6 months after her third Gardasil HPV vaccine booster. The patient experienced adverse manifestations shortly after the first dose of Gardasil injection (i.e. dizziness spells, paraesthesia and memory lapses) which were further exacerbated after the 2nd vaccine booster after which she also developed excessive tiredness (indicative of chronic fatigue), night sweats, loss of ability to use common objects, intermittent chest pain and sudden unexpected 'racing heart'. Although the autopsy examination failed to identify any toxicological, microbiological or anatomical cause of death, further investigations carried by Dr. Lee[39] showed that the post-mortem blood and splenic tissues tested positive for HPV-16 L1 gene DNA fragments corresponding to those previously found in 16 separate Gardasil vials from different vaccine lots (suspected to represent contaminants from the vaccine manufacturing process). These findings suggested that the quadrivalent HPV vaccine was indeed the most probable causal factor in this particular case. Specifically, the HPV DNA fragments detected in Gardasil vials appeared to be firmly bound to the aluminium adjuvant used in the vaccine formulation and thus likely protected against enzymatic degradation by endogenous nucleases.[40]

Additionally, thus far HPV vaccination has been linked to several autoimmune diseases, including Guillain-Barré syndrome,[41] other demyelinating neuropathies,[42 44] systemic lupus erythematosus,[3] pancreatitis,[45] vasculitis,[46] thrombocytopenic purpura[47] and autoimmune hepatitis.[48] Of note, the most prevalent adverse events associated with HPV vaccines appear to be autoimmune neurological diseases.[49,50] For instance, Sutton et al.[42] reported five cases of female patients who developed a multifocal or atypical demyelinating syndrome within 21 days of immunization with the quadrivalent HPV vaccine. As hypothesized by the authors, the temporal association with demyelinating events in these cases may be explained by the potent immune-stimulatory properties of HPV virus-like particles which comprise the vaccine. Similarly, Chang et al.[51] reported two cases who developed CNS demyelination closely following the administration of the HPV vaccine. Acute disseminated encephalomyelitis in young women (15 and 17 years old) within 3–8 weeks after HPV vaccination has also been described.[52,53] Altogether, these observations led to the hypothesis that the HPV vaccine may have been released too quickly into the market, in the absence of rigorous safety evaluations.[49,54,55] Indeed, Gardasil appears to have failed to meet a single one of the four criteria required by the FDA for Fast Track approval.[54]

Adjuvants in HPV Vaccines and Assessment of HPV Vaccine Safety in Clinical Trials

One of the most commonly used adjuvant in vaccines is aluminium[24] which is also present in HPV vaccines. There are two different brands of the HPV vaccine: the quadrivalent Gardasil (MSD) and the bivalent Cervarix (GSK). Both are composed of HPV L1 proteins that self-assemble to form virus-like particles but differ in the use of adjuvants.[56] While the first contains only aluminium hydroxyphosphate sulphate, the second contains a combination of an oil-based adjuvant monophosphoryl lipid A (MPL) and aluminium hydroxide (a proprietary brand of the vaccine manufacturer otherwise known as ASO4), thus leading to diverse boosts in immune responses between the two vaccines.[57] Another difference is the medium in which the vaccines are produced, *Trichoplusiani* cells for the Cervarix and *Saccharomyces cerevisiae* for the Gardasil. This distinction is even more intriguing because we know the potential of yeast to trigger autoimmune responses.[58] Nonetheless, a recent large observational study on the safety of the quadrivalent HPV vaccine allegedly identified no autoimmune safety concerns.[59] However, several important biases might have contributed to the negative findings of the study. Firstly, the study included all women who received at least one dose of the vaccine, thus making this particular population less sensitive for the detec-

tion of serious adverse reactions (given that such events occur with much lesser frequency when fewer doses of the vaccine are administered). Secondly, the research team failed to recruit appropriate expertise for diagnosis of autoimmune disorders. Namely, no immunologist/autoimmunologist, neurologist and ophthalmologist were present during the initial screening of the study participants which is particularly surprising in view of the fact that autoimmune conditions of interest that were examined included rheumatological, autoimmune disorders and neurological/ophthalmic conditions.[29,59] Finally, the Safety Review Committee failed to take into account the fact that autoimmune manifestations may be non-specific and not fitting a well-defined autoimmune condition[9,25,28] yet severely disabling.[26,35,60] Of note, the study was entirely funded by the quadrivalent HPV vaccine manufacturer Merck and all authors received previous founding from Merck and/or were consultants for the HPV vaccine manufacturer.[59]

Finally, a further major bias in evaluating HPV vaccine safety comes from the fact that in all clinical trials for both Gardasil and Cervarix, safety outcomes were compared between vaccine recipients and those who received an aluminium adjuvant containing 'placebo'.[49,50] This practice is common in vaccine trials,[61] despite much evidence showing that aluminium in vaccine relevant exposures can be toxic to humans,[34,35,60] and therefore, its use as a 'placebo control' in vaccine trials can no longer be justified.[61]

Conclusions

We documented here the evidence indicating the potential of the HPV vaccine to trigger a life-disabling autoimmune-mediated condition such as POF. Given that persistently infected women with HPV seem not to develop cancer if they are regularly screened and that the long-term clinical benefits of HPV vaccination are still a matter of speculation, a more rigorous assessment of vaccine risks and benefits is recommend.[49,50,62] Thus, physicians should remain within the rigorous rules of evidence-based medicine, to adequately assess the risks versus the benefits of HPV vaccination.[63,64]

Disclosure

An informed consent has been received from the patients present their cases. Y Shoenfeld has served as an expert witness in cases involving adverse vaccine reaction in the no-fault U.S. National Vaccine Injury Compensation Program. LT, SC and CP declare no conflict of interests. The authors thank the Dwoskin Family Foundation for support.

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Drug Saf (2013) 36:393–412
DOI 10.1007/s40264-013-0039-5

REVIEW ARTICLE

Safety of Human Papillomavirus Vaccines: A Review

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Published online: 1 May 2013

Abstract Vaccination to prevent human papillomavirus (HPV)-related infection leading to cancer, particularly cervical cancer, is a major public health breakthrough. There are currently two licensed HPV vaccines, both of which contain recombinant virus-like particles of HPV types 16 and 18 (which account for approximately 70 % of cervical cancer). One vaccine also protects against HPV types 6 and 11, which cause genital warts. The safety profile of both vaccines was assessed extensively in randomised controlled clinical trials conducted prior to licensure and has been further elucidated following licensure from surveillance and specific studies in large populations. This review aims to examine current evidence regarding the safety of HPV vaccines. In summary, both vaccines are associated with relatively high rates of injection site reactions, particularly pain, but this is usually of short duration and resolves spontaneously. Systemic reactions have generally been mild and self-limited. Post vaccination syncope has occurred, but can be avoided with appropriate care. Serious vaccine-attributable adverse events, such as anaphylaxis, are rare, and although not recommended for use in pregnancy, abnormal pregnancy outcomes following inadvertent administration do not appear to be associated with vaccination. HPV vaccines are used in a three-dose schedule predominantly in adolescent females: as such case reports linking vaccination with a range of new onset chronic conditions, including autoimmune diseases, have been made. However, well-conducted population-based studies show no association between HPV vaccine and a range of such conditions. Whilst this reassuring safety profile affirms the positive risk benefit of vaccination, as HPV vaccine use expands into more diverse populations, including males, ongoing safety assessment using well-conducted studies is appropriate.

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1 Introduction

HPV infection is a necessary step in the pathogenesis of cervical, other anogenital and some non-genital cancers [1, 2]. Primary prevention of infection with oncogenic HPV types has the potential to prevent morbidity and mortality worldwide. Cervical cancer alone is the fourth most common cause of cancer-related death worldwide [3, 4]. In addition to causing cancer, HPV infection also causes genital warts, the most common sexually transmitted disease in many developed country settings [5–7].

Two HPV vaccines are available: the bivalent vaccine Cervarix® (2vHPV, GlaxoSmithKline Biologicals, Belgium), which protects against the two oncogenic HPV types 16 and 18 that account for ~70 % of cervical cancer, and the quadrivalent vaccine Gardasil® (4vHPV, Merck and Co., USA), which protects against 16 and 18, as well as the non-oncogenic types 6 and 11, predominantly

responsible for genital warts and other non-malignant lesions. HPV antigens in both vaccines are composed of L1 proteins specific to each HPV type, which are derived using recombinant technology (yeast or insect cell in vitro expression systems) and form conformationally intact non-infectious virus like proteins (VLPs). The vaccines also contain adjuvants, which assist in enhancing the humoral immune response. For 4vHPV the adjuvant is a proprietary aluminium hydroxyphosphate sulphate system and for 2vHPV the adjuvant system is called AS04 and contains both an aluminium salt and monophosphoryl lipid A. [8, 9].

The clinical efficacy of these vaccines in the prevention of persistent HPV infection and intraepithelial neoplasia (potentially pre-cancerous lesions) at the cervix in women has been demonstrated in pre- and post-licensure studies [10–12]. Following impressive clinical trial results in females, both vaccines were made available for use from 2006–2007, with the 4vHPV vaccine now registered in 127 countries and an estimated >95 million doses distributed worldwide (Dr Carlos Sattler, personal communication, Merck, July 2012) and 2vHPV registered in >115 countries with >33 million doses distributed (Kristin Verschueren, personal communication, GSK, July 2012). Recently, population-based vaccination of males using 4vHPV has also been recommended in countries such as the USA, Canada and Australia [13–16] based on efficacy of this vaccine against vaccine-type persistent infection and intraepithelial neoplasia in anogenital sites in males [17]. The widespread availability of these vaccines with expanding use into new settings, particularly less developed countries, underpins the importance of reviewing the body of evidence for safety. Although there is no a priori reason to expect that the safety of HPV vaccines would be particularly different to other inactivated vaccines[18], concerns regarding perceived HPV vaccine safety issues have at times received extensive media attention and have the potential to reduce vaccine uptake [19, 20]. This review aimed to assess all available published safety data on both HPV vaccines, including randomised clinical trials, meta-analyses and data from post-licensure studies.

2 Methods

Articles cited in this review were obtained by searching OVID Medline (1946–May 2012) and OVID EMBASE (1980–May 2012) databases up to May 2012. Individual searches were completed on HPV vaccine safety, HPV vaccine trials and post-licensure studies, and HPV vaccines and pregnancy. Both database controlled vocabulary terms and commonly used free-text terms for HPV vaccines and/or safety were used: a full list of all search terms is available on request. There was no language restriction; however, only articles with English language (abstract or more) were reviewed. The bibliographies of identified articles and reviews were hand-searched to identify additional studies. Internet search engines were also queried for 'HPV vaccine case reports'. Formal inclusion and exclusion criteria were not applied for the purposes of selecting studies for the review; however, the authors endeavored to cite all studies (or pooled analyses) that contained original data, irrespective of the study type (e.g. controlled trial, case report, etc). Position statements and immunisation guidelines were also reviewed.

3 Pre-Licensure Controlled Clinical Trials and Long-Term Follow-Up Studies

The pre-licensure studies examining the safety of both 4vHPV and 2vHPV vaccines were extensive. In addition, a number of controlled clinical studies have continued past their pre-specified completion dates, with long-term follow-up of safety as well as immunogenicity/efficacy outcomes. Additional randomised controlled studies have been conducted in new populations in Korea, China, Japan and Vietnam [21–25]. Safety endpoints in most studies included local and systemic adverse events (AEs), serious AEs (SAEs), death and new onset medical conditions, including chronic and/or autoimmune disease. Pregnancy outcomes were also assessed and are discussed separately below. Many results presented below are from pooled analyses, which give the advantage of including large numbers of participants. Despite ongoing efforts to harmonise the categorisation and reporting of vaccine safety outcome measures in clinical trials [26], limitations on combining results from studies with different designs and/or outcome measures exist. Nevertheless, the results for most studies within these analyses were generally consistent.

3.1 Summary for 4vHPV from Clinical Trials

A pooled analysis on several early and pivotal trials [27–31] involving a total of >20,000 females aged 9–26 years and about 1,350 males aged 9–16 years, mostly from Europe, North and Latin America, showed that injection site reactions (ISR) were significantly more common in vaccine recipients compared with recipients of aluminium-containing placebo or non- aluminium placebo injections [32]. Injection site pain was most common (83 vs. 77 vs. 49 %, respectively, for the 3 recipient groups) with severe pain reported in 4 % of recipients compared with 2 % receiving aluminium-containing placebo. Erythema (24 versus 18 or 13 %) and swelling (24 versus 16 or 8 %) were also more common in vaccine recipients. Common systemic adverse experiences did not differ markedly

between groups, with headache most common (26 %), followed by fever (13 %) and nausea (6 %) [32].

In this analysis there was no significant difference in the frequency of SAEs overall or by system organ class (9 groups) over a median follow-up period of 3.6 years and >34,000 person-years-at-risk [32]. The five reported vaccine-related SAEs were not of any particular cluster [32]. Deaths occurred in 0.1 % of both vaccine and control recipients, respectively, with no death deemed related to the vaccine study [32]. The overall proportion reporting new onset autoimmune conditions was not different between both groups (2.4 % in each); specific conditions that were nominally higher among 4vHPV vaccine recipients included thyroiditis, rheumatoid arthritis and proteinuria, although each occurred in <0.1 % [32]. However, given the relative rarity of these individual conditions, even analysis of pooled data from many clinical trials does not provide enough participants to detect meaningful differences in such low-incidence events (discussed further in Sect. 5).

Another pooled analysis of trials in approximately 6,000 Latin American females aged 9–24 years also found more ISR in vaccine compared with placebo recipients (85 versus 73 %). Proportions with any systemic reaction (~60 %) and SAEs (<0.5 %) were comparable between vaccine and placebo recipients [33]. Smaller studies in females from Mexico and Korea reported similar frequencies of local and systemic AE [24, 34]. An open-label study in Vietnam comparing four 4vHPV vaccine dosing schedules found slightly lower rates of injection site pain (<69 % for any vaccine dose/schedule) [22]. With respect to age, in one trial a significantly lower proportion of younger (10–15 years) compared with older (16–23 years) females reported injections site pain (79 versus 86 %) and redness (20 versus 26 %). However, more younger females reported fever (12 versus 7 %) [27]. In a study of 4,000 older women (24–45 years) safety was comparable to younger females, with modestly lower frequencies of both local and systemic reactions [35, 36].

The 4vHPV vaccine was evaluated in a head-to-head comparison study between boys and girls aged 10–15 years in which local and systemic events were comparable between genders [27]. In one study of 4,000 males aged 16–26 years, ISRs were significantly more common among 4vHPV vaccine compared with aluminium-containing placebo recipients (60 versus 54 %) [37]; however, severe pain was uncommon in either group [17]. These frequencies were lower than reported among females in trials of similar design. Reporting of SAEs and new medical conditions was comparable between male vaccine and placebo recipients [17, 37], and safety in men who have sex with men, a subset of all males in these studies, was consistent with that for the whole study population [37]. Among subjects seropositive at baseline for at least one vaccine HPV type, the proportions reporting injection site reactions were similar to that of all subjects, and in both females and males [28, 29, 37].

When specifically reported, the frequency and severity of AEs following subsequent 4vHPV vaccine doses appeared similar, if not reduced, compared with AEs after the first dose [28, 29]. Two small studies in young females noted a decreasing proportion of recipients reporting injection site pain but increasing proportions reporting erythema and swelling with subsequent doses [27, 30]. Another study reported fewer participants with fever after each dose (5.1, 4.3 and 2.6 % following doses 1, 2 and 3 respectively) [24]. In males there was no increase, and suggestion of some decrease, in ISR and systemic adverse events with successive doses [37]. One small study reported a small increase in reactogenicity when more than three doses of vaccine were given; adverse events occurred in 80 % of women after a fourth dose at month 60 compared with 65.5 % after dose 3 at month 6. However, the majority of AEs were mild to moderate [38].

Studies of concomitant vaccination have generally demonstrated no or little change in the AE profile for the 4vHPV vaccine [30, 39–41]. For example, adverse reactions in adolescents given the 4vHPV vaccine concurrently with the hepatitis B vaccine, or alone, were comparable [41]. A modestly higher proportion of adolescents (4 %) reported ISR from 4vHPV and some systemic reactions (8 % more with headache) after concurrent administration of 4vHPV vaccine with the diphtheria-tetanus-acellular pertussis-inactivated poliomyelitis (dTpa-IPV) vaccine [40].

3.2 Summary for 2vHPV from clinical trials

The safety profile from clinical studies of the 2vHPV vaccine appears generally consistent with that for 4vHPV vaccine. A pooled analysis of 11 studies in about 30,000 females aged ≥10 years (with approximately 16,000 receiving at least one dose of 2vHPV and almost 46,000 vaccine doses administered) showed a higher incidence of ISR (pain, redness, swelling) in 2vHPV vaccine recipients compared with controls given an aluminium-containing control vaccine or hepatitis A vaccine [42]. The published trials within this review reported similar safety outcomes [10, 21, 25, 43–53]. Pain was the most common symptom (approximately 80 %) in all age groups and occurred in up to 97 % of adolescent girls [51]. Severe pain was reported in up to 7.5 % of recipients aged 15–25 years and severe ISR was more common in vaccine recipients (up to 0.6 % for redness and 1.2 % for swelling versus 0.1 and 0.2 %, respectively) [42]. However, ISRs were transient, generally lasting <5 days and mostly 2–3 days [10, 21, 25, 43–53].

Up to 55 % of 2vHPV vaccine recipients reported systemic symptoms, most commonly fatigue, headache and myalgia. Selected systemic symptoms were more common in vaccine recipients compared with control recipients in some, but not all, studies. There were no significant differences in SAEs, unsolicited symptoms and medically significant conditions [42]. Five deaths were reported (1 in a 2vHPV vaccine recipient and 4 in control recipients). However, no deaths were considered related to vaccination [42]. In follow-up studies, over 4 or more years, rates of vaccine-related SAEs, new-onset chronic disease and new-onset autoimmune disease were no different between groups [10, 44, 54, 55]. In addition, the proportions reporting significant medical events were similar among groups when stratified by age [56].

The safety profile of 2vHPV from smaller studies in new settings/populations has been consistent with earlier large studies, albeit with some limitations due to small numbers of participants. These have included studies in Korean girls (aged 10–14 years) [25] and women[46], a phase I study in 30 females in China [23] and a study in 50 girls aged 9–13 years in Bangladesh [45].

The frequency of ISR following 2vHPV vaccination, as well as systemic symptoms, appeared to diminish with increasing age among females [51], although in one study the AE profile was similar in those aged 10–14 and 15–25 years, respectively [49]. One study compared the three-dose schedule of the 2vHPV vaccine with two-dose schedules using the standard or double-strength formulations of the 2vHPV vaccine among women aged 9–25 years [57]. The AE profile over a 2-year follow-up period did not differ between the four groups, including those receiving the higher antigen content vaccine doses [57]. There is only one published trial of the use of 2vHPV vaccine in males and this did not include direct comparison with females. However, the AE profile reported in males was consistent with that from female studies [50].

Rates of AEs following concurrent administration of the 2vHPV vaccine with other vaccines (diphtheria-tetanus-acellular pertussis-inactivated poliomyelitis vaccine, diphtheria-tetanus-acellular pertussis vaccine, meningococcal conjugate vaccines, hepatitis B vaccine and the combined hepatitis A/hepatitis B vaccine) were generally comparable to those of participants given the 2vHPV vaccine alone [58–61] and similar to those for the 2vHPV vaccine administered alone in other studies. There was no increase in the frequency of solicited local or systemic adverse events with subsequent doses in the three-dose course of the 2vHPV vaccine, irrespective of whether the vaccine was administered alone [42, 48, 49] or concurrently with other vaccines [58, 61].

The 2vHPV vaccine contains a unique adjuvant called ASO4. A study investigating autoimmune diseases in all individuals enrolled in randomised, controlled trials of ASO4-containing vaccines ($n = 68{,}512$) over a mean follow-up period of 21 months found an overall rate of around 0.5 % for autoimmune events that did not differ between the AS04 and control groups [62]. The relative risk of an autoimmune event was 0.92 (95 % CI 0.70, 1.22) for the HPV 16/18 vaccine specifically.

3.3 Meta-Analyses of Clinical Trials

A number of meta-analyses of safety data from clinical trials of HPV vaccines have been conducted. One study [63] included data from six randomised controlled trials conducted up to June 2007 involving 2vHPV [44, 48, 52] and 4vHPV vaccines [12, 28, 29, 31], as well as the prototype monovalent type 16 HPV L1-VLP vaccine [64] in comparison with various controls. In the 40,323 female participants aged approximately 15–25 years, the incidence of SAEs in the vaccine and control groups was similar (odds ratio 0.998, 95 % CI 0.87–1.14). From four major studies that reported on death following HPV vaccine administration (two each for 4vHPV and 2vHPV), a total of ten deaths occurred in vaccine recipients and 11 deaths in control recipients (odds ratio 0.91, 95 % CI 0.39–2.14). No deaths were considered attributable to vaccination [63]. A more recent meta-analysis [65] that pooled data from seven unique clinical trials [10, 12, 28, 29, 31, 36, 44, 48, 52, 64, 66–68] involving 44,142 females given either 2vHPV or 4vHPV (or control/placebo) vaccines found no increase in the risk of SAEs among vaccine recipients (risk ratio 1.00; 95 % CI 0.91–1.09). There was a trend, which did not reach statistical significance, toward an increased risk of injection site-related SAEs among vaccine compared with control recipients (risk ratio 1.82; 95 % CI 0.79–4.20) [65].

3.4 Comparing 2vHPV Vaccine and 4vHPV Vaccine Based on Clinical Trials

One observer-blinded randomised head-to-head study compared 2vHPV and 4vHPV vaccines directly [69]. In the approximately 1,100 women aged 18–45 years, solicited AEs were significantly more common in 2vHPV compared with 4vHPV vaccine recipients: 95.1 % (95 % CI 92.8–96.7 %) versus 85.1 % (95 % CI 81.8–88.1 %), respectively [69]. Injection site pain, redness and swelling, and fatigue and myalgia were more common in 2vHPV vaccine recipients, as was severe ISR [17.4 % (95 % CI 14.2–20.9 % for 2vHPV) and 3.4 % (95 % CI 2.0–5.4 %) for 4vHPV, respectively]. However, overall most AEs were transient and of mild or moderate severity. At 24-month follow-up, the proportions reporting SAEs, significant medical conditions, new onset chronic diseases or autoimmune diseases were similar between the two groups

[70]. Of note, and possibly related to the propensity for more ISR following 2vHPV, the magnitude of the immune responses to each vaccine across all age strata was generally greater for 2vHPV. For example, geometric mean titres of serum neutralizing antibodies were 2.3–4.8-fold higher for HPV-16 and 6.8–9.1-fold higher for HPV-18 after 2vHPV compared with 4vHPV [69]. The higher ISR rates among 2vHPV vaccine recipients seen in this study needs to be considered against this evidence for better immunogenicity, although the clinical significance of the latter is not known. The safety results of this single head-to-head study are generally consistent with that from separate studies of both vaccines.

In summary, all randomised clinical trials of both 2vHPV and 4vHPV vaccines provide evidence of an excellent safety profile. Although both vaccines were associated with relatively high rates of ISR, particularly pain in most participants, when compared with either control vaccine or ISR reported for other routinely administered vaccines, these reactions were predominantly of short duration and resolved spontaneously. Systemic adverse events, such as headache and malaise, did not consistently occur more often in HPV vaccine recipients and the incidence of serious adverse events, including new onset chronic conditions and deaths, was not different in HPV vaccine recipients than in controls subjects. Whilst these data are robust, there are limitations inherent in clinical trials and meta-analyses. They are not powered to assess differences in specific chronic and/or autoimmune conditions, proposed as being potentially vaccine-related, and are not conducted in persons wholly representative of the population (for example by ethnicity or underlying medical conditions). As such, post-licensure studies in large populations have been important (discussed in Sect. 5.4).

4 Safety of HPV Vaccines During Pregnancy

HPV vaccines are not recommended for use in pregnant women and pregnant subjects were excluded from participating in the clinical trials of both HPV vaccines. However, some participants did become pregnant despite using contraception and undergoing pregnancy testing prior to dose administration. The relatively large number of pregnancies that occurred, the prospective nature of the studies, control groups and documentation of outcomes make these clinical data very valuable [71]. Pregnancy outcomes for vaccine trial participants were reported irrespective of the relationship between conception and dose/s, but also separately for participants who conceived within either 30 or 90 days of vaccination. For the majority of pregnancies, vaccination did not occur during the period when any theoretical risk would be most biologically plausible (close to the time of conception or during early embryogenesis). Thus, these studies are still limited by their small sample size. Following vaccine registration, pregnancy registries for both vaccines have been established [72, 73]. These registries collect spontaneously reported cases of exposure, which can assist in detection of a significant concern. However, they also have many limitations, such as limited knowledge of precise gestational age at time of exposure, selective reporting, underreporting, inability to calculate reporting rates (in vaccinated and unvaccinated individuals) and representativeness (in comparison with the overall pregnant population).

4.1 The 4vHPV Vaccine and Pregnancy

Overall, the proportions of pregnancies that resulted in an adverse outcome (spontaneous abortion, late foetal death, infant with congenital anomalies) among the 4vHPV vaccine and control recipients who became pregnant at any time during the clinical trials course (13–16 % of >12,000 participants aged 15–26 years) were similar [74]. In one study, where 70 women were vaccinated within 30 days of conception, there were 5 cases of congenital abnormalities in infants of vaccine recipients compared with none among 66 women who received aluminium-containing placebo, with a statistically significant risk difference (4.5; 95 % CI 1.1–10.1) [28]. However, those abnormalities were relatively common and unrelated: hip dysplasia, congenital ankyglossia/congenital pyloric stenosis, congenital hydronephrosis, congenital megacolon and talipes. A subsequent pooled analysis of five phase 3 clinical trials, for outcomes following vaccination within 30 days of conception, identified no additional cases among vaccine recipients but one infant with congenital anomalies born to a placebo recipient. This rendered the risk difference statistically insignificant [74]. Over all time periods, the number of infants with congenital anomalies was not statistically different between the vaccine and placebo groups (40 versus 30; 2.0 versus 1.5 %, $p = 0.20$) and rates were consistent with those expected. Although generally reassuring, caution in drawing firm conclusions from this pooled analysis is warranted, in part because of various methodological limitations, particularly post-hoc pooling of studies of different design [71].

Post-licensure data on pregnancy outcomes reported to the pregnancy registry for 4vHPV established by the manufacturer are published covering a 2-year period [75]. These included 517 prospectively followed pregnancies, with 451 (87.2 %) resulting in live births, including three sets of twins [72]. Of these HPV vaccine-exposed neonates

439 (96.7 %) were normal. The rates of spontaneous abortions [6.9 per 100 outcomes (95 % CI 4.8–9.6)] and major birth defects were not greater than unexposed population rates. The prevalence of major birth defects was 2.2 per 100 live-born neonates (95 % CI 1.05–4.05). The registry also received reports of two cases each of the rare conditions anencephaly and schizencephaly (one reported prospectively and one retrospectively for each condition) in infants whose mothers received the vaccine within 2–21 days after the last menstrual period [72]. The estimated population prevalence of these two conditions was 1.1 per 100,000 births and 1.5 per 100,000 births, respectively, but because of the limitations discussed above, incidence rate estimates in the vaccinated population cannot be calculated. Detailed annual reports from this registry can be obtained on request. The most recent contains data on approximately 1,500 pregnancies prospectively followed to May 2011 and does not indicate a causal relationship between 4vHPV and adverse pregnancy outcomes [75].

4.2 The 2vHPV Vaccine and Pregnancy

Similar to 4vHPV, there are no specific studies of the use of 2vHPV in pregnant women. Published studies reporting on pregnancy outcomes in 2vHPV clinical trial participants predominantly focussed on miscarriage (spontaneous abortion) rates. A combined analysis from two double-blind randomised controlled trials of 2vHPV found that among >26,000 women aged 15–25 years there were 3,599 pregnancies eligible for analysis [76]. The 2vHPV vaccine was not associated with an increase in the risk of miscarriage compared with the control hepatitis A vaccine (11.5 versus 10.1 %, respectively), and rates of miscarriage were not different from that expected. In women who conceived within 90 days after receiving a vaccine or placebo dose ($n = 230$), a non-significant increased rate in miscarriage in the 2vHPV vaccine recipients was observed (13.7 versus 9.2 %, $p = 0.033$ by permutation test) [76].

Another pooled analysis, which included participants of one of the two studies above, found that in 415 participants who became pregnant around the time of vaccination spontaneous abortion rates varied between 2vHPV and control recipients, being higher among younger 2vHPV vaccine recipients [11 % compared with those who received aluminium-containing placebo (8.3 %) or hepatitis A vaccine (5.8 %)], but lower among older 2vHPV compared with placebo recipients [42]. Rates of pregnancy resulting in premature delivery did not differ substantially. Data on congenital anomalies were not reported in these published studies [42]. A vaccine registry for 2vHPV [77] has also been established, but no published data from the registry are available.

4.3 Conclusion Regarding Use of HPV Vaccines in Pregnant Women

In summary, pregnancy adverse outcomes in both 4vHPV and 2vHPV studies appeared similar overall among vaccine compared with control recipients and were comparable to population rates. Evidence for an epidemiologic association of infant congenital anomalies or miscarriage with receipt of a dose of either vaccine at any time, including close to conception, has not been established from the available clinical trial and post-licensure data. However, as discussed above, these studies have limitations, and additional data from population-based data linkage studies of pregnancy outcomes in vaccinated women, would be valuable. For example, a study of ASO3-adjuvanted pandemic influenza vaccine in Denmark demonstrated no difference in pregnancy outcomes in vaccinated compared with unvaccinated women [78]. Immunisation guidelines recommend that pregnant women should avoid vaccination until after delivery [16, 79, 80]. Women inadvertently vaccinated whilst pregnant, or who conceive shortly after vaccination, can be reassured there is no evidence to indicate need for medical termination of pregnancy [16, 79].

5 Post-Licensure Experience

Although safety is one of the primary outcomes measured in vaccine clinical trials, the number of participants included and short follow-up periods are limitations [81] that inherently restrict the identification of rare adverse events [18]. Clinical trial participants are often homogenous with regards to age, ethnicity and health status and hence trial results are not always generalisable to the populations in which vaccines will be introduced. Post-licensure surveillance is essential to detect rare or unexpected adverse events and to monitor safety in large diverse populations under variable real-life conditions. Many post-licensure assessments of HPV vaccine safety have been performed and are discussed below [18, 82].

5.1 Passive Surveillance

Some countries have passive reporting systems for AE related to medicines and vaccines in which information is spontaneously reported by health practitioners or the public rather than systematically sought out. These include the UK Yellow Card scheme (medicines and vaccines) [83], and others specifically for vaccines, including the US Vaccine Adverse Event Reporting System (VAERS) [84], and the Canadian Adverse Events Following Immunisation Surveillance System (CAEFISS) [85–87]. Reports of

adverse events following immunisation (AEFI), in addition to medicines, are also collected via Vigibase, the World Health Organisation's programme, from multiple countries [88]. However, these systems have inherent limitations including: capture of only a small proportion of total adverse events; great variation in reporting frequency, quality and completeness; lack of timely accurate data on vaccine usage (estimates of incidence rates calculated on all doses distributed rather than by age stratified data on doses administered); lack of detailed clinical data required to assess causality; and recording of all events independent of whether a causal association with the vaccine administered exists [89]. Identification of safety signals for unexpected or previously unknown events may arise [90]; however, determining causality is often difficult [91] and usually requires further evaluation using additional surveillance and epidemiological studies [92].

Published national passive surveillance data for HPV vaccination are available from Australia, the Netherlands, USA and UK [93–96]. As expected, reporting rates differ (Table 1) because of differences in reporting mechanisms, case definitions and how rates are derived. For example, by the end of 2008, the US VAERS received 12,424 reports of adverse events following more than 23 million doses of the 4vHPV vaccine distributed, giving an overall reporting rate of 539 reports per million doses distributed [94]. In comparison, reporting rates for adverse events following 4vHPV in Australia were 249 per million doses distributed, but were higher (~400 per million) if limited to school girls only [97, 98]. With 2vHPV, higher reporting rates for adverse events were seen in the UK (1,045 per million doses administered) and in the Netherlands following a vaccination campaign for girls 12–16 years of age (1,160 per million doses administered); however, this may represent system differences rather than differences in AE rates per se. [95, 99]. Reporting rates cannot be calculated for data from multiple countries using WHO's Vigibase because of the lack of data on doses distributed or administered [99].

The majority of AEFIs with the HPV vaccination reported via passive surveillance have been minor and align with expected adverse events seen in pre-licensure clinical trials. The most commonly reported AEFI included injection site reactions, headache and dizziness [93–96, 100]. Only a small and expected proportion of AEFIs were categorised as 'serious', for example, 7 % of all reports to VAERS [101]. The majority of reported events to date are in females: in the 2 years after the 4vHPV vaccine was registered in males in the US, there were 504 male reports made to VAERS (6.5 % considered serious). The most common non-serious adverse events reported by males were similar to females and included dizziness, syncope and injection site pain [101].

A myriad of more serious adverse events following HPV vaccination have been reported to passive surveillance systems, including anaphylaxis, Guillain-Barré syndrome, transverse myelitis and thromboembolic events [94]. Comparisons of observed versus expected incidence rates of disease in a population are subject to the limitations of the passive reporting systems discussed above and the availability of data on background rates for a specific condition. Thus, enhanced passive and/or active surveillance to detect specific adverse events and/or to investigate signals derived from passive surveillance have been conducted or are ongoing. As discussed below, such studies utilise accurate data on vaccines administered and can better establish risk of HPV-vaccine attributable AE [82].

No deaths reported and published in passive surveillance systems data have been attributed as causally related to either of the HPV vaccines [93, 96, 101]. The importance of thoroughly investigating deaths that occur shortly after vaccination to determine potential alternative causes was highlighted by a much publicized case in the UK, where a teenage girl died suddenly on the same day of vaccination from a previously undetected tumour [102]. The propensity to assume causality with serious outcomes was also poignantly highlighted when a HPV demonstration project in females in India was suspended because of the occurrence of four deaths temporarily related to vaccination [103]. Although the deaths have been reportedly found to be unrelated to vaccination, loss of confidence in HPV vaccination led to the suspension of clinical studies of HPV vaccine in India [104].

5.2 Case Reports and Case Series

When a vaccine is first licensed, publications of AEFI reports from individuals or groups of individuals are common. However, such 'case reports' can only rarely provide strong evidence of a causal link with vaccination, typically when the AE that occurs is directly related to the vaccine (for example, injection site reactions or isolation of a neuropathic vaccine-derived poliovirus from a recently vaccinated patient with paralytic poliomyelitis). Even if such reports, together with other scientific data, present credible "mechanistic" evidence of the possibility of a vaccine causing the adverse event, the frequency at which these events occur in relationship to vaccination or in the absence of vaccination (that is, the epidemiologic evidence) will not be available from such studies [105].

Table 2 lists published case reports/case series of AEs following HPV immunisation. Of these, five describe a case or cases of local/regional reactions related to the injection site. Such reports may highlight the occurrence of vaccine delivery errors, the need for improvement in vaccine administration techniques and/or important

Table 1 Reporting rates for adverse events following HPV vaccination from passive safety surveillance systems

Period	Surveillance system/setting	Vaccine	Measured outcome	Reporting rates per 100,000 doses distributed (unless otherwise specified[a,c])	References
Australia					
April 2007–June 2010	TGA (national)	4vHPV	Any AEFI	24.9[b]	[93]
			Anaphylaxis	0.26	
			Fainting	2.2[b]	
April 2007–August 2009	TGA (national)	4vHPV	Any AEFI	24.0	[136]
			Anaphylaxis	0.25	
2007	School-based cohort (2 states: Vic, South Australia)	4vHPV	Hypersensitivity (suspected)	9.2[a,b] (all rates per doses administered)	[98]
			Hypersensitivity (probable)	0.8[a,b]	
			Anaphylaxis	0.5[a,b]	
2007	School-based cohort (1 state: NSW)	4vHPV	Any AEFI	41.0[a] (all rates per doses administered)	[97]
			Anaphylaxis (suspected)	4.5[a]	
			Anaphylaxis (probable)	2.6[a]	
May 2007–April 2009	SAEFVIC (1 state: Victoria)	4vHPV	Any AEFI	40.4[b]	[127]
			Fainting	7.8	
2010 and 2011	12–17 year olds (1 state: NSW)	4vHPV	Any AEFI	14.1 (2010)[a] (all rates per doses administered)	[137, 138]
				37.9 (2011)[a]	
Europe					
2009	RIVM (national, The Netherlands)	2vHPV	Any AEFI	116.0[a] (all rates per doses administered)	[95]
			Fainting	10.0[b]	
			Anaphylaxis	No cases reported	
April 2008–July 2010	Yellow cards (national, UK)	2vHPV	Any AEFI	104.5[a] (all rates per doses administered)	[96]
			Psychogenic events (including fainting)	19.8[a,b]	
			Anaphylaxis	1.0[a,b]	
USA					
June 2006–Dec 2008	VAERS (national, USA)	4vHPV	Any AEFI	53.9	[99]
			Fainting	8.0[b]	
			Anaphylaxis (serious only)	0.03[b]	
June 2006–Sept 2009	VAERS (national, USA)	4vHPV	GBS	0.80[a]	[131]
			GBS (6 weeks after vaccination)	0.07[a,c]	
June 2006–December 2008	VAERS (national, USA,)	4vHPV	Any AEFI	53.9	[94]
			Fainting	8.2	
			Anaphylaxis	0.1	
			Hypersensitivity	3.1	

[a] Reporting rate calculated based on number of vaccine doses administered

[b] Reporting rates calculated based on values provided in manuscript

[c] Weekly reporting rates (per week per 100,000 patients)

Table 2 Published case series/case reports of adverse events following HPV immunisation

References	Country	Vaccine	Condition	Summary of case details	Comments
Local/regional reactions					
[139]	USA	4vHPV	Unilateral cervical and supraclavicular lymphadenopathy	A 26-year-old woman day 3. Same side as injection and no other symptoms/signs. Dose 1 only	Contemporaneous VAERS search by authors found 55 non-confirmed reports of lymphadenopathy post HPV vaccine
[140]	Belgium	4vHPV	Brachial plexus neuritis	A 19-year-old woman onset 1 month post dose 2 same side as vaccination. Surgery opposite wrist 2 months prior	Contemporaneous VAERS search by authors found 4 non-confirmed reports post HPV vaccine
					Aetiology unknown. Associated with surgery, infection, autoimmune disease and vaccination in preceding 3–14 days
[141]	USA	4vHPV	Aluminium granuloma	A 26-year-old woman, tender nodule outer left arm 2 months post dose 3	History of granulomatous appendicitis, raises question of alternative aetiology for granulomatous disease
[107]	Australia	4vHPV	Localised lipoatrophy	Two cases aged 23 and 25 vaccinated with three doses over 6 months	Contemporaneous VAERS search by authors found 11 non-confirmed reports of injection site atrophy post HPV vaccine
					Uncommon condition. Often idiopathic but can be due to local trauma, inflammatory conditions and injections of drugs including whole cell pertussis vaccine
[106]	Australia	4vHPV	Complex regional pain syndrome	Four females (aged 12–16 years) developed persistent pain in extremity/ies disproportionate to inciting event (vaccination)	Uncommon condition. Syndromic diagnosis. Behavioural and physical therapy optimal
					Time to complete resolution varied from 5 days–7 months
Systemic reactions					
[20]	Australia	4vHPV	Mass psychogenic response	26 school girls sought care in their school clinic after vaccination for a variety of symptoms (dizziness, palpitations, weakness). 4 girls presented to hospital emergency department	None identified to have organic cause after extensive medical investigation
[142]	Australia	4vHPV	Pancreatitis	A 26-year-old woman 4 days post dose 1	Pancreatitis incidence 25 per 100,000 in women 25–29 years in Australia
				No cause identified	Multiple causes, 10 % idiopathic
[143]	Austria	2vHPV	Acute disseminated encephalomyelitis (ADEM)	A 15-year-old girl 23 days post dose 2	
[109]	Australia	4vHPV	Acute disseminated encephalomyelitis (ADEM)/multiple sclerosis	Five patients aged 16-25 with clinical presentation of ADEM or MS between 1 to 21 days post dose 2 or 3	Three patients had prior demyelination episodes
					MS incidence 6 per 100,000 in women 20-29 years in Australia. Peak in young women
[144]	Germany	4vHPV	Acute disseminated encephalomyelitis (ADEM)	A 20-year-old woman within 28 days of dose 2	

Table 2 continued

References	Country	Vaccine	Condition	Summary of case details	Comments
[145]	USA	4vHPV	Acute disseminated encephalomyelitis (ADEM)	A 16-year-old 10 days post dose 2	
[146]	USA	N/S	Fulminant myocarditis	A 17-year-old 1 week post dose 1	Major cause of sudden death in children and young adults
[147]	USA	4vHPV	Multiple sclerosis first presentation	Two young women approx. 1 month post vaccine	
[148]	USA	4vHPV	CNS demyelination. First presentation of MS	Two women aged 19 and 18. Onset 1 month post dose 2 and 6 weeks post dose 1	
[149]	USA	4vHPV	Postural tachycardia syndrome	A 20-year-old 2 weeks post dose 3	Pathogenesis uncertain, may have autoimmune aetiology. Increased prevalence in young women
[150]	USA	4vHPV	Ampiginous choroiditis	A 17-year-old 3 weeks post dose 1	Rare bilateral aggressive posterior uveitis
[151]	USA	4vHPV	Opsoclonus myoclonus	An 11-year-old 15 days after dose 1 developed moodiness. One month later eye symptoms developed. Deterioration 4 days post dose 2 with ataxia and myoclonus	Rare neurological condition. Paraneoplastic syndrome in 50–60 % cases. Autoantibodies detected that target the cerebellum
[152]	Italy	2vHPV	Telogen effluvium	Two patients 11 years of age with loss of hair post dose 2. Worsened post dose 3 then gradually resolved	
[153]	Italy	2vHPV	Bilateral papilloedema	An 11-year-old girl 10 days post dose 2	Laboratory studies did not support vaccination as the cause
[154]	France	4vHPV	Immune thrombocytopenic purpura	A 16-year-old 3 months post dose 2	
[155]	Philippines	N/S	Systemic lupus erythematosus	Three cases reported: a 17-year-old with onset 2 months post dose 2; 45 year old (past history of rheumatoid arthritis) 4 months post dose 2. 58 year old flare of long-standing SLE 3 months post dose 2	

N/S vaccine type not specifically stated, however, presumed to be 4vHPV

Table 3 Post-licensure studies of adverse events following HPV vaccine using enhanced passive surveillance methods

Population/setting	Vaccine	Methods of evaluation	Outcome reported after HPV	References
The Netherlands 2009	2vHPV	Web-based survey on ISR and systemic reactions within 7 days after each vaccine dose	92 % reported ISR (post dose 1)	[156]
HPV catch-up cohort girls aged 13–16 years ($n = 4,282$)		74 % overall response rate (29 % for all 3 doses)	Lower odds of ISR after dose 2 and 3 compared with dose 1 (OR 0.33; 95 % CI 0.28–0.38 and OR 0.43; 95 % CI 0.37–0.51, respectively)	
			92 % reported systemic AE post dose 1, especially myalgia, fatigue and headache. Lower proportion post doses 2 and 3. Systemic AE more frequent in older girls	
			Girls with headache/cold/flu symptoms prior to vaccination had increased rates of ISR and systemic AEs	
			15 % used analgesics post dose 1	
			1.5 % consulted a medical practitioner post dose 1	
			No hospitalisations occurred	
Nepal 2008 secondary school girls aged 10–26 years ($n = 1,096$)	4vHPV	Not stated	7.8 % reported pain at injection site (with any dose)	[113]
Italy 2008–2010 cohort of girls aged 12–26 years in two regions ($n = 4,643$)	2vHPV	Questionnaire-based survey (66 % response rate for dose 1, lower for subsequent doses)	68 % reported pain at injection site	[114]
			26 % reported fatigue	
			17 % reported headache	
			0.5 % reported urticaria ($n = 33$)	
			Syncope (9/7,107 = 1.3 per 1,000 doses)	
			No serious AEs reported	
USA 2008 females aged 11–26 years post dose 1 ($n = 899$)	4vHPV	Questionnaire on AE and attitudes (27 % response rate)	78 % reported pain at injection site	[115]
		Data extracted from electronic medical records	1 % reported syncope, 15 % "pre-syncope"	

Table 4 Post-licensure population-based studies of adverse events following HPV vaccine

Setting/ period	Vaccine	Study cohort	Measured outcome	Main finding	References
UK/ Ireland 2008–2009	2vHPV	Children <16 years with suspected anaphylaxis following vaccination reported to British Paediatric Surveillance Unit (BPSU) >2 million doses administered	Anaphylaxis following HPV (and other) vaccines	Low incidence estimated at 1.4 cases/million doses	[116]
France 2007	2vHPV/ 4vHPV	~5.8 million adolescents and young women; 15.6 % had received ≥1 dose of HPV vaccine (French national healthcare insurance database)	8 pre-specified autoimmune disorders[d]	No signal for autoimmune disorders with HPV vaccination	[123]
USA 2006–2008	4vHPV	~190,000 females aged predominantly 9–26 years who received at least one dose of HPV vaccine (2 managed care organisations)	16 pre-specified autoimmune conditions with new onset within 180 days post each HPV vaccine dose[a]	No evidence of safety signal for autoimmune conditions following 4vHPV vaccination. The IRR for Hashimoto's thyroiditis was significantly elevated but further investigation suggested no relationship[b] Vaccination with 4vHPV associated with syncope occurring on the same day of vaccination and 'skin infections' (likely representing ISR) within 2 weeks following vaccination. No other safety concerns detected	[119]
USA 2006–2008	4vHPV	~190,000 females aged predominantly 9–26 years who received at least one dose of HPV vaccine (2 managed care organisations)	Emergency department visits and hospitalisations within pre-defined risk periods for multiple conditions detected using 265 diagnostic codes		[122]
USA 2006–2009	4vHPV	Females aged 9–26 years, administered >600,000 HPV vaccine doses (7 managed care organisations)	Eight pre-specified well-defined and severe adverse events following HPV vaccine[c]	No statistically significant increased risk for any of the pre-specified adverse events detected Possible association with VTE in girls 9–17 years, however, all confirmed cases had other risk factors	[120]
USA 2006–2009	4vHPV	Females aged 9–26 years in US Vaccine Safety Datalink (VSD) (9.2 million people annually in 8 managed care organisations)	Eight pre-specified well-defined and severe adverse events following HPV vaccine[c]	No statistically significant increased risk for any of the pre-specified adverse events detected Potential signals identified for HPV vaccine with appendicitis and allergic reactions in first 2 week of data collection. RR decreased to null in later weeks	[121]
USA 2005–2008	4vHPV	Retrospective cohort of 12.6 million adolescents/young adults (11–21 years) in five US healthcare plans	Guillain-Barré syndrome following meningococcal conjugate vaccine Secondary outcome: GBS after other vaccines, including 4vHPV	Two cases of GBS observed within 6 weeks of HPV vaccine (584,458 doses) compared with 0.37 expected. Could not exclude risk, although study not designed to make definitive conclusion for HPV vaccination	[117]
USA 2000–2009	4vHPV	1.8 million children aged up to 17 years (5 managed care organisations)	Immune thrombocytopenic purpura (ITP) following different vaccines	No elevated risk following HPV vaccination	[118]

[a] The conditions were: (1) rheumatologic/autoimmune disorders, including immune thrombocytopenia (ITP), autoimmune haemolytic anaemia, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and juvenile rheumatoid arthritis (JRA), (2) autoimmune endocrine conditions, including type 1 diabetes, Hashimoto's disease and Graves' disease and (3) autoimmune neurological/ophthalmic conditions, including multiple sclerosis (MS), acute disseminated encephalomyelitis, other demyelinating diseases of the central nervous system, vaccine-associated demyelination, Guillain-Barré syndrome, neuromyelitis optica, optic neuritis and uveitis

[b] Onset of thyroiditis symptoms occurred prior to vaccination in a number of patients who had received a recent HPV vaccine

[c] Pre-specified adverse events were: Guillan-Barré syndrome (GBS), stroke, venous thromboembolism (VTE), appendicitis, seizures, syncope, allergic reactions and anaphylaxis. Pre-specified post-vaccination periods of risk varied by condition

[d] Eight pre-specified autoimmune disorders (rheumatologic, endocrinologic and neurologic conditions) were considered but not stipulated in the abstract

management issues, for example, the need to avoid repeated vaccination into the same site at which lipoatrophy has occurred or to institute prompt multi-modal therapy for complex regional pain syndrome [106, 107]. However, other reports describe occurrences of serious or striking disease onset for conditions whose aetiology and/or pathogenesis is uncertain. It is well recognised that reporting and attribution of such cases to vaccination can be anticipated when a new vaccine is introduced into a population, particularly where large-scale programmes with high coverage and multiple vaccine doses are implemented [108]. However, implicating vaccines as a causal factor for such diseases, for example multiple sclerosis [109], requires more than a temporal association. Analysis of disease rates between vaccinated and unvaccinated individuals, a consistent temporal relationship, biological plausibility and lack of alternative aetiology are essential factors to be considered [105, 108]. Population-based studies and other evidence available to date, discussed more below, provide evidence that autoimmune diseases are not triggered by HPV vaccination.

Table 2 also contains reference to other events found not to be biologically related to vaccination, such as the occurrence of a "mass psychogenic" response to vaccination among girls in a school-based vaccination clinic in Australia. This report highlighted that factors other than the vaccine constituents, such as the fear of painful events, can trigger adverse reactions to vaccination [20, 110]. Expert review of unusual and serious conditions that occur after vaccination, such as that conducted in specialised immunisation adverse event assessment clinics, is important [111, 112].

5.3 Enhanced Passive and Active Surveillance

Table 3 describes four post-licensure studies that used survey methods to investigate adverse reactions following HPV vaccination predominantly in teenage girls in four different settings: the Netherlands and Italy (2vHPV) and the USA and Nepal (4vHPV). The quality of these studies, particularly the response rate, varied. Overall, the rates of ISR and systemic reactions reported following vaccination were similar to that seen in randomised controlled clinical trials, with the exception of the limited data from the study in Nepal [113], where only 8 % of girls reported pain at the injection site following 4vHPV. The methods of data collection were not presented in this study and it appears likely that methodological and socio-cultural factors may have limited perception and/or reporting of pain.

The most comprehensive study was in the Netherlands, where 4,248 girls aged 13–16 years (74 % of those approached) responded [95]. Participation rates declined progressively after the first, second and third doses of vaccine were received; however, overall no unexpected or SAEs were reported (Table 3). Reporting rates for ISRs were comparable to those for the 2vHPV vaccine in clinical trials, although a higher proportion of participants subjectively reported "pronounced pain" at the injection site (24, 12 and 15 % by dose, respectively). ISRs and systemic symptoms were significantly less common after second and third doses, although this did not account for factors such as non-completion of vaccine course or the survey. Girls who reported feeling unwell in the week prior to vaccination, or who had a history of certain chronic medical conditions, had a statistically higher likelihood of reporting AEs post-vaccination. In a similar study among Italian school girls, no SAEs were reported [114]. ISRs, myalgia, fatigue and headache were the most common reported symptoms. Pain was more commonly reported after the first dose, whereas rates of other local and systemic reactions were higher after subsequent doses. AEs were less frequently reported than in published clinical trials. A smaller study from the US, with a lower response rate but utilising medical record review to also ascertain AE, found girls reported similar ISRs compared with clinical trial data [115]. This study was notable for high rates ($n = 134$, 15 %) of syncope and dizziness (recorded as "pre-syncope") after vaccination.

5.4 Population-Based Epidemiologic Surveillance

Table 4 lists seven published studies of SAE following HPV vaccination in well-defined large populations. Three of these studies focussed on specific conditions: anaphylaxis [116], Guillain-Barré syndrome [117] and thrombocytopenia [118] in children following vaccination of any type. The other five publications report on observational cohort studies conducted using large healthcare databases in the USA [119–122] or France [123] and examined the incidence of pre-specified new onset conditions following vaccination. The study from France (on 2vHPV vaccine) was presented in abstract format only and could not be fully scrutinised. In the US studies, potential new onset conditions were identified by various methods (including hospital/emergency department discharge diagnosis codes, pharmacy prescriptions and laboratory data) and cases had in-depth clinical review. Comparison with incidence rates in unvaccinated same-age female populations (concurrently or historically) or rates following vaccines other than 4vHPV were made. Two studies report on females aged 9–26 years between August 2006 and October 2009 in the Vaccine Safety Datalink (VSD) cohort, in which a total of 600,558 doses of 4vHPV vaccine were recorded [120, 121]. There was no statistically significant increased risk in vaccine recipients for the outcomes studied [these included GBS, stroke, appendicitis, seizures, allergic reactions,

anaphylaxis, syncope and venous thromboembolism (VTE)]. The other two US studies [119, 122] were based on a cohort of almost 190,000 females aged 9–26 years who had received at least one dose of the 4vHPV vaccine. There was no cluster in the onset of 16 pre-specified new onset autoimmune diseases (NOAID) over an 180-day period in relationship to vaccination, age or dose(s) received [119]. Further investigation of an elevated incidence rate ratio for one of the 16 conditions, Hashimoto's disease, did not suggest a true association, as many of the cases were likely pre-existing at the time of vaccination. A more extensive review of medical attendance for 265 distinctly coded conditions occurring in various plausible risk periods following vaccination indicated that only syncope (on the day of vaccination) and 'skin infections' (likely representing injection site reactions) occurred more commonly after vaccination than during non-exposure periods [122].

With the introduction of new vaccines such as HPV vaccines, where three doses are scheduled over a 6-month period, there will inevitably be the onset of new conditions, including immune-mediated diseases, associated in time with vaccination [124]. Rates of immune-mediated diseases in the female population targeted for HPV vaccination (9–26 years) are relatively high, with disease incidence varying substantially across this age group [124]. For example, the hospitalisation rate for autoimmune conditions in women 19–30 years is up to 389/100,000 [62], and in US women aged 19–28 years diagnoses of thyroiditis and multiple sclerosis are ten times more frequent than in adolescent girls [108]. Studies to determine "background rates" of diseases suggested to be potentially vaccine-attributable have been conducted in some countries, such as Denmark [125] and the US [62, 108], and are key to understanding and addressing vaccine safety concerns when they inevitably arise.

5.5 Conditions of Interest Arising from Post-Licensure Surveillance

5.5.1 Syncope

Disproportionately higher rates of syncope after HPV vaccination compared with that observed in pre-licensure RCTs were reported from post-licensure surveillance in the US [115], Australia [94], the Netherlands [126] and Italy [114]. The reporting rate for syncope (Table 1) following 4vHPV was similar in the USA and the Netherlands and in state-based but not national reports from Australia, at approximately 80–100 per million doses [127]. Although neither dizziness nor syncope was reported at increased rates in vaccine compared with control recipients in pre-licensure studies, given the high overall reporting rate of pain following vaccination, syncope in response to this painful stimulus is not unexpected under real-life conditions. It has become clear that in the adolescent vaccine target population, reports of syncope following any vaccination are common compared with other target groups [128]. In the US, syncope in persons >5 years of age following any vaccination increased from 0.28 per million vaccine doses distributed in 2004 to 0.54 per million doses distributed in 2006 after the introduction of three new vaccines targeted to adolescents: meningococcal conjugate vaccine, reduced dose diphtheria-tetanus-pertussis vaccine and HPV vaccine [129]. Analysis of VSD data found no increased risk of syncope specifically following HPV4 when compared with other vaccines given to adolescents [120]. Syncope can lead to syncopal seizures [127] and falls: about 15 % of syncopal episodes reported to VAERS resulted in a fall, of which 68 % resulted in a head injury [94]. This highlights the importance of practical measures to anticipate and/or avoid syncope and its complications, such as lying down during, and resting after, vaccination [128, 129]. In one Australian review, a majority of patients with HPV vaccine-related syncope or syncopal seizures were re-vaccinated without recurrence [127].

5.5.2 Anaphylaxis

Although considered a rare AEFI, anaphylaxis can occur because of antigen, vaccine adjuvant and/or vaccine excipients [98]. Of note, no cases of vaccine-related anaphylaxis were reported in the phase 3 studies of HPV vaccines, although episodes of immediate and severe allergic reactions (facial oedema, severe bronchospasm) occurred [18]. Reporting rates of anaphylaxis following HPV vaccination have generally been consistent in both national passive surveillance systems and population-based studies (Tables 1, 3) and within the estimated range of that for other vaccines of 1–10 cases per million doses. The exception was one early study from Australia in which higher rates of anaphylaxis (2.6 per 100,000 doses) were reported in a school-based program [97]; this could partly be explained by the different surveillance mechanism and case definitions employed in this study. Of note, the 4vHPV vaccine is produced in a yeast-based (*Saccharomyces cerevisiae*) system and use of this vaccine is contraindicated in persons with a history of immediate hypersensitivity to yeast. The 2vHPV vaccine pre-filled syringes contain latex within the stopper and should not be used in persons with a history of anaphylaxis to latex [130]. Overall, the evidence from post-licensure surveillance suggests that anaphylaxis and serious allergic reactions following HPV vaccination are rare and manageable, consistent with that found for other vaccines. In two retrospective cohort studies in females from Australia, only a proportion of those reported as 'suspected' anaphylaxis

cases were classified as anaphylaxis on clinical review, and even fewer (1/19) were found to have probable hypersensitivity after skin prick testing [97, 98]. The majority of girls with a history of suspected anaphylaxis who were re-vaccinated under close medical observation had no subsequent adverse reactions [98].

5.5.3 Guillain-Barré syndrome (GBS)

A review of GBS cases reported to the US VAERS estimated a 2.5- to 10-times greater rate of GBS within 6 weeks after 4vHPV vaccination compared with that expected in the general population (6.6 events per week per 10 million subjects versus 0.65–2.57 cases per week per 10 million population) [131]. However, the increased propensity to report events that occur in a temporal relationship to vaccination and lack of a control group meant further investigation to better delineate any possible relationship was required. Subsequent population-based studies, employing extensive case finding methods for GBS, have not provided evidence of a rate that is significantly greater than that expected in the adolescent and young adult female population (Table 4) [119, 120].

5.5.4 Venous Thromboembolism (VTE)

A safety signal for VTE was identified following review of VAERS reports to June 2006 [94]. However, of the cases that had sufficient information for review, 90 % had a known risk factor for VTE, such as oral contraceptive use. The time to onset after vaccination was also highly variable [94]. Analysis of VTE in the VSD population-based cohort revealed a non-significant increased relative risk of 1.98 among females aged 9–17 years when compared with historical rates; however, all five cases had known risk factors for VTE [120]. Increased rates of VTE post HPV vaccination have not been reported in other settings, suggesting this is unlikely to be causally related to vaccination.

6 Summary of Findings

A variety of population-based post-licensure studies have assessed adverse events, including those with delayed onset, in females given HPV vaccines. Despite the inevitable publication of case reports raising the potential association between HPV vaccination and a range of severe chronic conditions of poorly defined aetiology such as multiple sclerosis, evidence from these well-conducted population-based studies has consistently not identified any new or concerning safety issues. Randomised controlled clinical trials conducted before vaccine licensure identified that ISR (particularly pain) and mild self-limited systemic symptoms (such as myalgia and headache) occur commonly after HPV vaccination and should be anticipated. One head-to-head comparison study found injection site reactions and some systemic symptoms to be more common in 2vHPV compared with 4vHPV vaccine recipients although, overall, events in most recipients were well-tolerated and self-limiting. Allergic reactions and syncope can occur following HPV vaccination but can be managed with appropriate care [132]. Data from pooled clinical studies of HPV vaccination given inadvertently during pregnancy or near the time of conception have not provided evidence for foetal harm or higher rates of miscarriage; however, advice regarding avoiding vaccination during pregnancy is warranted, given the limitations of such studies.

The findings of this review are consistent with the expert assessment of a number of peak advisory bodies [13, 96, 133]. For example, the Global Advisory Committee of the World Health Organisation found in 2007 and 2008 that current evidence on the safety of HPV vaccines was reassuring [132, 134]. Independent systematic reviews of HPV vaccine safety have also drawn this conclusion [18]. In addition, a recent report by the Institute of Medicine (IOM) summarising evidence for causal relationships between certain adverse events and eight different vaccines, including HPV vaccines, identified acceptable levels of evidence to support a causal relationship between HPV vaccination and anaphylaxis and reported acceptance of a causal relationship with any vaccine injection and syncope [105]. Consistent with the findings of this review, the IOM stated that neither the mechanistic or epidemiologic evidence supported an association of HPV vaccination with other outcomes, such as various new onset chronic diseases.

7 Conclusions and Future Considerations

This overview provides an update of the continually expanding body of evidence regarding the safety profile of the two currently licensed HPV vaccines, 2vHPV (Cervarix®) and 4vHPV (Gardasil®). Both have been well characterised in extensive clinical trials and a range of post-licensure studies, some of the most comprehensive employed for any vaccine. Overwhelming, the evidence supports an excellent safety profile, not unlike the majority of other inactivated vaccines assessed in similar populations.

Ongoing initiatives to assess vaccine safety have been summarised elsewhere [82, 135]. Despite the reassuring evidence to date, safety studies in developing countries that adopt HPV vaccine use, and in specific populations not extensively assessed to date, such as males, are awaited. For example, studies in immunocompromised persons, an

important target group, are limited and should occur. In parallel, research to better elucidate background rates of serious diseases and events that can be coincidentally associated in time with vaccination are essential to inform decision-making around vaccine safety issues as they arise [124] and to avoid a loss of confidence in these potentially life-saving vaccines.

Acknowledgments The authors wish to thank Dr. Rashmi Dixit, formerly of the National Centre for Immunisation Research and Surveillance (NCIRS), for assistance in reviewing some studies. We also thank other NCIRS staff for their assistance: Ms. Catherine King for literature searches and Mr. Edward Jacyna and Ms. Lyn Benfield for assistance with data collection and collation.

Dr. Brotherton was a partner investigator on an Australian Research Council (ARC) Linkage Grant for which CSL Biotherapies was a partner organization

Associate Professor Macartney, Dr. Clayton Chiu and Dr. Melina Georgousakis are employees of the Australian National Centre for Immunisation Research and Surveillance, which receives funding from the Australian Government Department of Health and Ageing and the New South Wales Department of Health. Dr. Brotherton is an employee of the Victorian Cytology Service (VCS), which receives funding from the Victorian Health Department and the Australian Government Department of Health and Ageing.

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Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Case Line Listing

You searched for the following:

Report Date: 27/11/2019,14/01/2020,5/02/2020,17/02/2020,30/09/2020,9/04/2021
Reaction Outcome: Fatal
Study Type: Other studies,Unknown
Case Decision: Accepted,Rejected,Withdrawn
Characterisation: Suspect,Interacting
Trade Name: Gardasil,Gardasil 9

Number of Reports: 6

Limitations of the data

This document contains information from reports of adverse events that the TGA has received in relation to therapeutic goods. It does not contain all known information, and an assessment of the safety of a medicine cannot be made based on this information.

Causality

- The reports received by the TGA contain suspected associations that reflect the observations of an individual reporter. The reporter may be a health professional, a sponsor, or a member of the public.
- Adverse events are suspected of being related to a therapeutic good, but this relationship is usually not certain - the symptom may be related to the underlying illness or to other factors.
- There might be no relationship between the adverse event and the medicine - it may be a coincidence that the adverse event occurred when the medicine was taken.

The information provided in the following table is from reports included in the Adverse Event Management System (TGA's internal adverse event database). Cases in the DAEN are indicated by blue shading. The cases that do not appear in the Database of Adverse Event Notifications (DAEN) may have insufficient information and/or no reasonable temporal association and/or the relationship between the medicine/vaccine and the adverse event appears to not be related as judged by a health professional and/or the report may be a duplicate of a case included in the DAEN and/or the report may have been received by the TGA within the last 90 days.

Case no.	Report Entry Date	Gender	Age (yrs)	Medicine reported as being taken	MedDRA Reaction terms
[redacted]	27/11/2019	[redacted]	16	Gardasil (HPV Type 11 L1 Protein ; HPV Type 16 L1 Protein ; HPV Type 18 L1 Protein ; HPV Type 6 L1 Protein) - Suspect s22 [redacted]	Death; Ovarian cancer; Pyrexia
485949	14/01/2020	Female	-	Gardasil (HPV Type 11 L1 Protein; HPV Type 16 L1 Protein ; HPV Type 18 L1 Protein ; HPV Type 6 L1 Protein) - Suspect [redacted]	Amyotrophic lateral sclerosis; Asthenia; Autoimmune disorder; Dysstasia; Gait disturbance; Headache; Hypoaesthesia; Limb injury; Motor neurone disease; Multiple sclerosis; Muscle atrophy; Muscular weakness; Nausea; Obstructive sleep apnoea syndrome; Paraesthesia; Paralysis; Respiratory failure; Seizure; Spinal cord disorder; Tremor; Urinary incontinence
487445	05/02/2020	Male	12	Gardasil (HPV Type 11 L1 Protein; HPV Type 16 L1 Protein ; HPV Type 18 L1 Protein ; HPV Type 6 L1 Protein) - Suspect s22 [redacted] Trade Name Not Specified s22 [redacted] Concomitant [redacted]	Glioblastoma
488425	17/02/2020	Male	12	Boostrix (Diphtheria toxoid ; Pertactin ; Pertussis filamentous haemagglutinin ; Pertussis toxoid ; Tetanus toxoid) - Suspect [redacted] Gardasil (HPV Type 11 L1 Protein ; HPV Type 16 L1 Protein ; HPV Type 18 L1 Protein ; HPV Type 6 L1 Protein) - Suspect s22 [redacted]	Glioblastoma; Pyrexia
508309	30/09/2020	Male	12	Gardasil (HPV Type 11 L1 Protein ; HPV Type 16 L1 Protein ; HPV Type 18 L1 Protein ; HPV Type 6 L1 Protein) - Suspect s22 [redacted]	Acute disseminated encephalomyelitis

534593	09/04/2021	Female	12	Boostrix (Diphtheria toxoid ; Pertactin ; Pertussis filamentous haemagglutinin ; Pertussis toxoid ; Tetanus toxoid) - Suspect [redacted] Gardasil (HPV Type 11 L1 Protein ; HPV Type 16 L1 Protein ; HPV Type 18 L1 Protein ; HPV Type 6 L1 Protein) - Suspect [redacted] s22	Cardiomyopathy; Pulmonary artery thrombosis

Gardasil batch assessment 2007 - 2015

Batch No.

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0513F (NE43230)
NE58880
NE58880
NE13110 (US 0512F)
NF16830 (US 0513F)
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N2007 (US 0120Y)
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N3594 (US 1611X)
R2818 (US 0663Z)
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S1159 (US 0663Z)
S1378 (US 0239AA)

Australian Government
Department of Health
Therapeutic Goods Administration

Office of Laboratories and Scientific Services

Operations	
Procedure	Bacterial Endotoxin Testing - Appendix 14K - Kinetic LAL Standard Operating Procedure
Written	s22 [redacted]
Authorised	s22 [redacted]
Date issued	20 August 2014
Revision #	0

Purpose

The purpose of this document is to describe the Standard Operating Procedure used for testing the bacterial endotoxin content of vaccines and other parenterally administered medicines with the kinetic chromogenic method. The procedures contained within this document are performed in accordance with the harmonised methods of Ph. Eur. 2.6.14 and USP <85>. The results obtained using this procedure, including any deviations, will be documented in the appropriate worksheets.

Scope

The scope of this Standard Operating Procedure is limited to the Immunobiology Section of the Office of Laboratories and Scientific Services, TGA. Any changes to the procedure would need to be assessed to determine whether a re-validation is necessary.

Responsibility

Only valid operators from the Immunobiology Section, OLSS are to carry out this procedure.

The maintenance of this document is the responsibility of the Senior Scientist. Approval of any changes can be performed by this Officer or the Principal Scientist, Immunobiology

Before performing this procedure for routine assays, operators must be officially trained according to the Bacterial Endotoxin training sheets. Operators are required to pass an initial qualification (IQ) assay for the kinetic chromogenic method before performing further testing.

Background

The test for bacterial endotoxins uses a lysate of amoebocytes from the blood of the horseshoe crab, *Limulus polyphemus*. Gram-negative bacterial endotoxin triggers an enzyme cascade leading to the activation of a proenzyme in the Limulus Amoebocyte Lysate (LAL).

The addition of a solution containing endotoxins to a solution of the lysate produces turbidity, a chromogenic reaction or gelation of the mixture depending on the type of lysate preparation. The rate of reaction depends on the concentration of endotoxin, the pH and the temperature. The kinetic chromogenic lysate used in this assay contains certain divalent cations, proenzymes and a peptide substrate linked to the coloured product p-nitroaniline which is read at 405 nm. The presence of endotoxins in a product may be masked by factors interfering with these enzyme dependent reactions.

Prior to routine testing, the product to be tested must have been shown not to interfere with the assay. A test for interfering factors is conducted on 3 batches of the product (wherever possible) to determine the routine dilution at which no interference is observed. Please read the Bacterial Endotoxin Testing Manual for more information regarding the validation/qualification of the method for use with different products.

Limulus Amoebocyte Lysate (LAL)
The LAL Reagent, or lysate, should be reconstituted according to the manufacturer's instructions. Currently, using Lonza's KQCL kit, this involves reconstitution with 2.6 ml of LAL Reagent Water (LRW). This should only be done immediately before it is required for use because once reconstituted, background cleavage of the substrate can occur, resulting in very low levels of colour formation. Prolonged exposure to air, light and potential endotoxin contamination should all be avoided. The reconstituted LAL reagent is stable if stored protected from light at 2-8°C for 8 hours. Alternatively, it can be stored at or below -10°C for up to 14 days and thawed only once.

Care must be taken when reconstituting lysate to avoid frothing. The vial should be swirled gently or rolled. Shaking too vigorously can lead to the proteins denaturing, reducing enzyme activity and also causes bubbles in the reaction plate that will not allow the kinetic assay to be analysed correctly by the plate reader.

Control Standard Endotoxin (CSE)
The Control Standard Endotoxin CSE should be reconstituted according to the manufacturer's instructions. Currently, using Lonza's KQCL kit, this involves reconstitution with LAL Reagent Water (LRW). The endotoxin concentration is specific to each batch of CSE when matched to a specific lysate lot and calibrated against the international Reference Standard Endotoxin (RSE). The volume is different for each batch of CSE so that the vial is made up to the correct endotoxin concentration of 50 EU/ml. The correct volume to be added is available on the Certificate of Analysis from the Lonza website.

The CSE is lipopolysaccharide purified from bacterial cell walls. It is not easily maintained in solution and will readily adhere to the vial and to itself. For this reason all standard solutions must be mixed very thoroughly. The reconstituted vial is shaken vigorously on a vortex for at least 15 minutes before use. Once reconstituted the CSE must be stored at 2-8°C and used within 4 weeks (28 days). When using a vial that has been reconstituted previously, the vial must be equilibrated to room temperature and vortexed for 15 minutes prior to each use.

Standard Curve and Positive Product Controls
The CSE standard curve for **most** endotoxin testing on the WinKQCL system consists of 5 solutions, each being a 10 fold dilution of the previous, beginning with the reconstituted CSE. The concentrations are 50, 5, 0.5, 0.05 & 0.005 EU/ml and the Positive Product Control (PPC) spikes are usually set in the middle of the curve at 0.5 EU/ml.

The PPC spike is therefore usually a 10 µl aliquot of the 5 EU/ml standard pipetted directly into the appropriate empty wells of the assay plate. A 100 µl aliquot of each sample is then placed into the appropriate 4 wells of the assay plate for the test and PPC.

The assay is begun with the addition of lysate while incubating the plate at 37°C.

The use of a tube or bottle to contain the negative control (LRW) and the 50 EU/ml standard prior to dispensing to the plate, while strictly correct, is not usual practice for endotoxin testing.

Some products are tested using different sets of standards and/or different endotoxin concentrations for the PPC. For example, s22
s22

Bacterial Endotoxin Limit
This is the maximum allowable amount of bacterial endotoxin in a product, measured in either EU/ml or EU/mg. Some products have established endotoxin limits set in pharmacopoeial monographs. For other products, calculation of the limit can be performed using the following formula: Endotoxin Limit = K / M

Where: K is the maximum allowable pyrogenic dose (EU/kg/h)
M is the maximum recommended dose of the product (amount/kg/h)

Companies sometimes set endotoxin limits stricter than the pharmacopoeial or calculated values.

Maximum Valid Dilution (MVD)
The MVD is the value, calculated using the endotoxin limit of the product and the sensitivity of the assay, that the product can legitimately be diluted to for testing.

$$MVD = \frac{\text{Endotoxin Limit} \times \text{Product Concentration}}{\text{Lysate sensitivity } (\lambda)}$$

pH Values
The reactions that drive this assay are enzyme based and do not work as effectively outside a pH range of 6-8. The lysate is lyophilised (and therefore reconstituted) in a formulation that buffers the pH effects of most products. If there is a chance that the pH of the product under investigation may cause assay interference due to pH, then appropriate dilutions of the product added to lysate should be tested for pH.

Analyst and Lysate Qualification
An initial qualification (IQ) assay must be performed on each new batch of lysate. All analysts are required to perform this IQ assay before doing routine assays. Analysts who have not maintained their competency by performing at least one valid routine assay within a 12 month period are obliged to perform this IQ assay before doing routine assays.

References
European Pharmacopoeia 2.6.14 - Bacterial Endotoxins
United States Pharmacopoeia <85> - Bacterial Endotoxin Test
Brochure for kinetic chromogenic kit (Lonza Limulus Amoebocyte Lysate (LAL) Kinetic-QCL)
FDA Guidance for Industry – Pyrogen and Endotoxins Testing: Questions and Answers
Immunobiology Section Bacterial Endotoxin Manual R11/319706
Current Risk Assessment for bacterial endotoxin testing procedures

WHS Requirements
Section 10 of the Immunobiology Section Laboratory Operations Manual details the WHS aspects of our work. There are no extraordinary requirements specific for this assay. Appropriate PPE must be worn when performing this procedure. A risk assessment for bacterial endotoxin testing is found at http://tgalqs/riskassess/index.htm

From the “New and edit” drop down menu select Open/edit assessment. At the next page scroll down to find the applicable assessment.

Method
Equipment and Materials
Lonza Endotoxin Detection System – Biotek Plate Reader (ELX808) and WinKQCL software
Vortex mixer/s
Pipettors – 10 µl, 200 µl & 1000 µl adjustable pipettes and 100 µl multichannel pipette
* Must be within calibration period and be calibrated with the correct pyrogen-free tips
Pipette tips – currently Eppendorf Biopur purchased from and certified pyrogen-free by Lonza
Tubes - purchased from and certified pyrogen-free by Lonza
Reagent reservoir (purchased from and certified pyrogen-free by Lonza)
Pyrogen-free 13 x 100 mm borosilicate glass tubes (purchased or depyrogenated in-house)
Plates (purchased from and certified pyrogen-free by Lonza – currently Costar 3596)
Lonza Limulus Amoebocyte Lysate Kinetic-QCL Kit – containing LAL Reagent, Control Standard Endotoxin and LAL Reagent Water
Depyrogenated black lid screw-top bottles or re-used tubes (in-house)
Forceps, scissors, Parafilm

Using the contents of a Kinetic Chromogenic kit (currently Lonza's Limulus Amoebocyte Lysate (LAL) Kinetic-QCL), products are tested at their established routine dilution. A set of standards are run and each of the samples is run with a Positive Product Control (PPC). The PPC is used for every sample to ensure the absence of factors that may inhibit the enzymatic reaction.

Precautions
Ensure that the correct (**endotoxin free**) tips, tubes, plates etc are used.
Careful technique is required to avoid endotoxin contamination.
The times and temperatures used in this assay must be adhered to.

Print and fill out the appropriate paperwork from the Quality Management System.

The method should be carried out as per this SOP, which is based on the manufacturer's instructions in the Lonza KQCL kit insert, and on pharmacopoeial methods.

Please read ALL of this method before beginning the assay.

The outcome of the assay results depends heavily upon the timing and technique of adding the lysate to the reaction plate. This is especially evident when performing an Initial Qualification assay due to the quadruplicate nature of the assay. This means that the remainder of the assay method is largely about the preparation leading up to this important step.

Setting up the Software

- Turn on the plate reader and turn on and log on to the computer using your current Windows password. Double click on the WinKQCL4 desktop icon. Log in using the WinKQCL user ID and password set up with you by someone with Supervisor access.
- Click on the Templates tab and click New. To save the template please use the date, initials and assay type format as shown in the templates listed.
- Remove the kit reagents required for this assay from the cold room and allow to equilibrate to room temperature before use.
- If the CSE to be used has not yet been reconstituted, open Internet Explorer, and from the Lonza website download the Certificate of Analysis for the lot of kit reagents to be used in the assay. This document contains the reconstitution volume required to make an endotoxin concentration of 50 IU/ml.
- When a new template opens, the Setup tab is open. Under "Test Type" select the correct type of assay, eg. "Initial Qualification", or "Routine Assay".
- Click the "Lot/Exp" tab and enter the Lot Number and Expiration of the lysate, water and standard endotoxin to be used in the assay.
- You will also need to confirm the number of points on the standard curve. Most assays currently use 5 points (50 – 0.005 EU/ml). s22 s22
- Fill out the accessories to be used for the assay. (eg. pipettes, tips etc). The pipettors will need to be within calibration and the consumables within their expiry dates. For in-house accessories use the date bottles/tubes were placed in the hot air oven as the batch number.
- Save the template. The default name is today's date but can be changed here if required.
- Drag the Template manager screen out to allow the sample names to be read more easily. Click Print and select Plate Layout. It is easier to use if it is printed in "Landscape". The print out may help to set up the dilutions.

Assay Set-Up
Move to the lab bench to begin setting up the dilutions.
Ensure that the correct (pyrogen-free) tips are used. Obtain the required pipettes, reagent reservoir (keep in bag), dilution tubes or bottles, reaction plate, tip discard, sharps bin etc.

- Open the seal around the reaction plate and retain the plastic base of the seal. This can be used as a non-pyrogenic surface for temporary storage of the rubber stoppers for the LRW and Lysate vials that need to remain pyrogen free.

- Keep the lid on the plate and if desired, mark the assay on the lid of the plate as per the Template Plate Layout printed earlier.
- Carefully open the aluminium seal on the LRW vial and discard metal into a sharps bin.

Preparation of Control Standard Endotoxin
If there is already a reconstituted vial of CSE within the 28 day use-by date in the cold room, this step is not required. Stored reconstituted CSE must be vortexed for 15 minutes prior to use.

- Carefully remove aluminium seal on the standard endotoxin vial and discard metal to sharps bin.
- Carefully loosen the rubber stopper on the endotoxin vial to allow the vacuum to be released without contaminating the vial and without losing any of the vial contents.
- Open the endotoxin vial, resting the stopper upside down on the lab bench (**not** on the pyrogen free plate seal).
- Open the LRW vial, resting the stopper upside down in the pyrogen-free base of the plate seal.
- Reconstitute the endotoxin with the specified volume of LRW stated on the Certificate of Analysis.
- Lift the LRW stopper with forceps and replace on the LRW vial. The water will be used again later.
- Lift the CSE stopper with forceps, replace on the CSE vial and seal with parafilm. Label the vial with the reconstitution date.
- Place the vial of reconstituted CSE on the vortex and shake vigorously for at least 15 minutes.
- Preparation of the CSE should be recorded on a printed copy of Appendix 4K.

The next steps to be performed are dependent upon the type of assay, and this SOP has been divided into 3 types, Initial Qualification, Routine Assay & s22 [redacted]. Move to the most appropriate section below based on the type of assay you are performing.

- Rack and label the appropriate number of dilution tubes as required in the appropriate table below. If using pyrogen free test tubes, remove from the bottom of the foil packaging ensuring you do not introduce contamination. If using the black lid screw-top bottles or pre-used tubes ensure that they have been depyrogenated.
- The CSE dilutions are dispensed to the plate as they are prepared, in order to save mixing time.

Initial Qualification Assay
If performing an IQ assay, quadruplicate aliquots of the standards are added to the plate. Remove the rubber seal from the LRW and place it upside down onto the pyrogen-free surface of the plate container. Pipette LAL Reagent Water (LRW) into tubes as instructed below.

Standards for Initial Qualification (IQ) Assay

Concentration	Volume Water	Volume Endotoxin	Plate (Quadruplicate)
50 EU/ml	No tube required	-	F1 - F4
5 EU/ml	900 µl LRW	100 µl of 50 EU/ml	E1 - E4
0.5 EU/ml	900 µl LRW	100 µl of 5 EU/ml	D1 - D4
0.05 EU/ml	900 µl LRW	100 µl of 0.5 EU/ml	C1 - C4
0.005 EU/ml	900 µl LRW	100 µl of 0.05 EU/ml	B1 - B4
Blank	1 ml LRW	-	A1 - A4

- Immediately after removing the CSE from the vortex, open the vial, place the lid on the bench and slide back the lid of the plate. Pre-wet tip and dispense 100 µl of the 50 EU/ml CSE into the appropriate quadruplicate wells in the plate (F1-F4). It is preferable to do this directly into the base of the well, or touching the “difficult” side of the well, usually the right side for right handed people. The “easy” side will be used for lysate later. Replace plate lid. Using the same or a fresh tip, pipette 100 µl into the 5 EU/ml tube. Discard tip. Replace stopper on CSE vial.

- Vortex the 5 EU/ml tube for a timed 1 minute and slide back the lid of the plate. Immediately pre-wet tip and dispense 100 µl of the 5 EU/ml solution into the appropriate quadruplicate wells of the plate (E1-E4). Replace plate lid. Using the same or a fresh tip, pipette 100 µl into the 0.5 EU/ml tube. Discard tip.
- Vortex the 0.5 EU/ml tube for a timed 1 minute and slide back the lid of the plate. Immediately pre-wet tip and dispense 100 µl of the 0.5 EU/ml solution into the appropriate quadruplicate wells of the plate (D1-D4). Using the same or a fresh tip, pipette 100 µl into the 0.05 EU/ml tube and discard tip.
- Vortex the 0.05 EU/ml tube for a timed 1 minute and slide back the lid of the plate. Immediately pre-wet tip and dispense 100 µl into the appropriate quadruplicate wells of the plate (C1-C4). Using the same or a fresh tip, pipette 100 µl into the 0.005 EU/ml tube and discard tip.
- Vortex the 0.005 EU/ml tube for a timed 1 minute and slide back the lid of the plate. Immediately pre-wet tip and dispense 100 µl into the appropriate quadruplicate wells of the plate (B1-B4). Discard tip.
- Remove the rubber seal from the LRW and place it upside down onto the pyrogen-free surface of the plate container. Slide the lid of the reaction plate to expose row A. Dispense 100 µl of LRW into each of the wells in the plate for the Negative Control (A1-A4). Replace lid. Discard tip.

Since this is an IQ assay, move on to preparing the software and lysate.

Routine Assay

If performing a routine assay, duplicate aliquots of the standards are added to the plate.
Remove the rubber seal from the LRW and place it upside down onto the pyrogen-free surface of the plate container. Pipette LAL Reagent Water (LRW) into tubes as instructed below.

Standards for Routine Assay

Concentration	Volume Water	Volume Endotoxin	Plate ID (Duplicate)
50 EU/ml	No tube required	-	F1 – F2
5 EU/ml	900 µl LRW	100 µl of 50 EU/ml	E1 – E2
0.5 EU/ml	900 µl LRW	100 µl of 5 EU/ml	D1 – D2
0.05 EU/ml	900 µl LRW	100 µl of 0.5 EU/ml	C1 – C2
0.005 EU/ml	900 µl LRW	100 µl of 0.05 EU/ml	B1 – B2
Blank	1 ml LRW	-	A1 – A2

PPC is a 10 µl aliquot of the **5** EU/ml standard in a total of 110 µl (0.45 EU/ml)

- Immediately after removing the CSE from the vortex, open the vial, place the lid on the bench and slide back the lid of the plate. Pre-wet tip and dispense 100 µl of the 50 EU/ml CSE into the appropriate duplicate wells in the plate (F1-F2). It is preferable to do this directly into the base of the well, or touching the “difficult” side of the well, usually the right side for right handed people. The “easy” side will be used for lysate later. Replace plate lid. Using the same or a fresh tip, pipette 100 µl into the 5 EU/ml tube. Discard tip. Replace stopper on CSE vial.
- Vortex the 5 EU/ml tube for a timed 1 minute and slide back the lid of the plate. Immediately pre-wet tip and dispense 100 µl of the 5 EU/ml solution into the appropriate duplicate wells of the plate (E1 & E2). Replace plate lid. Using the same or a fresh tip, pipette 100 µl into the 0.5 EU/ml tube. Discard.
- Vortex the 0.5 EU/ml tube for a timed 1 minute and slide back the lid of the plate. Immediately pre-wet tip and dispense 100 µl of the 0.5 EU/ml solution into the appropriate duplicate wells of the plate (D1-D2). Using the same or a fresh tip, pipette 100 µl into the 0.05 EU/ml tube and discard tip.
- Vortex the 0.05 EU/ml tube for a timed 1 minute and slide back the lid of the plate. Immediately pre-wet tip and dispense 100 µl into the appropriate duplicate wells of the plate (C1-C2). Using the same or a fresh tip, pipette 100 µl into the 0.005 EU/ml tube and discard tip.
- Vortex the 0.005 EU/ml tube for a timed 1 minute and slide back the lid of the plate. Immediately pre-wet tip and dispense 100 µl into the appropriate duplicate wells of the plate (B1-B4). Discard.

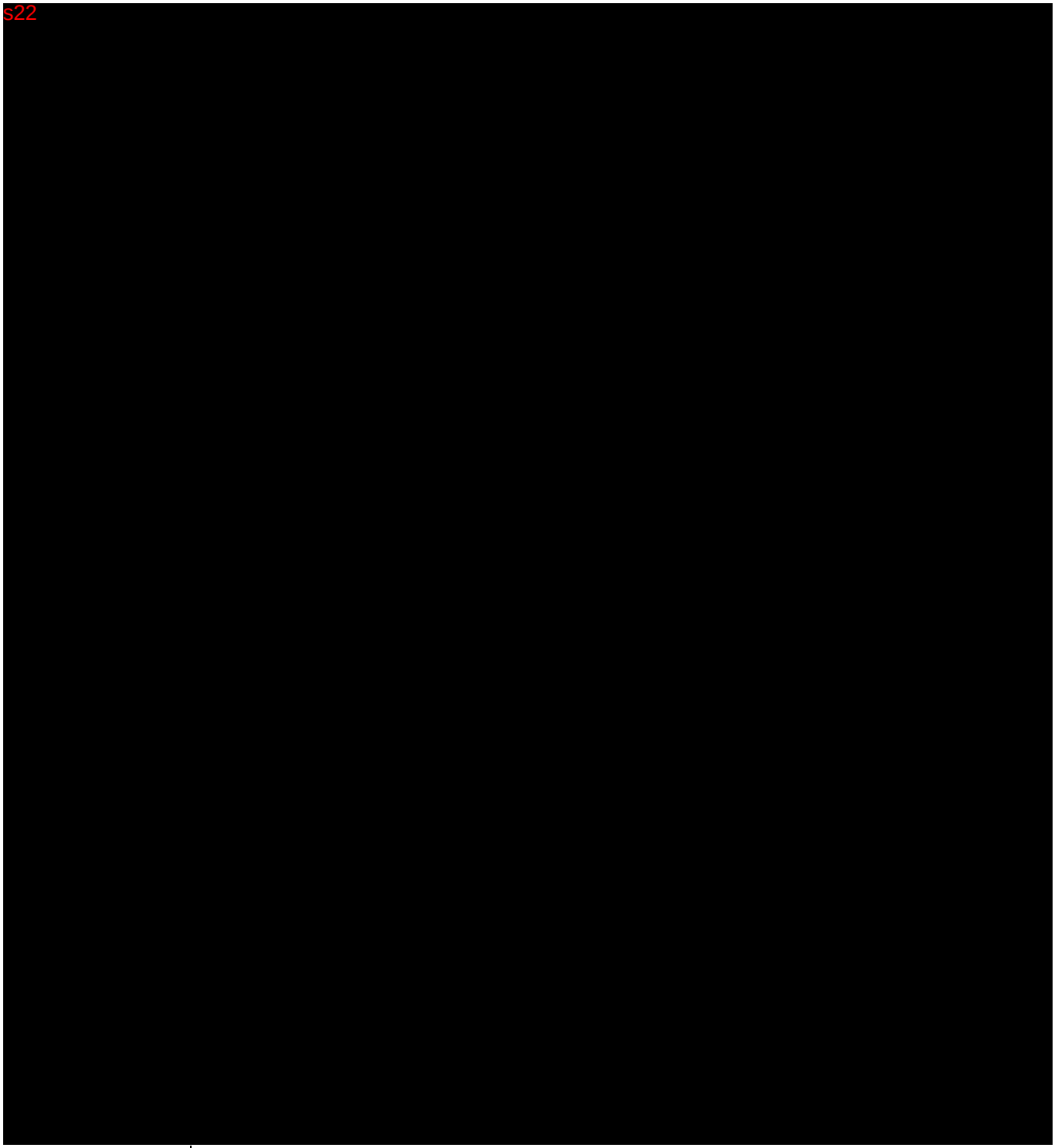
- Remove the rubber seal from the LRW and place it upside down onto the pyrogen-free surface of the plate container. Slide the lid of the reaction plate to expose row A. Dispense 100 µl of LRW into each of the wells in the plate for the Negative Control (A1-A2). Replace lid. Discard tip.

Dispense Positive Product Control

- For routine assays the PPC is usually a final concentration of around 0.5 EU/ml. This is achieved by adding 10 µl of the 5 EU/ml standard to the PPC wells. After the addition of 100 µl of sample, the theoretical endotoxin concentration is 0.45 EU/ml.

- Ensure that the 10 µl pipette is ready. Vortex the **5** EU/ml standard tube for a timed 1 minute. Take note of where the PPC wells are using the printed plate layout, or the lid of the plate. Slide back the lid of the plate and immediately pre-wet tip and dispense 10 µl of the **5** EU/ml solution into the appropriate duplicate PPC wells of the plate. If you have marked the plate lid it is a good idea to keep sliding the lid on and off between adding the PPC.

s22



s22 [redacted]

Preparation of Sample Dilutions
If you are performing an assay to test samples (using a Routine Assay or a [redacted]) you will also need to set up the appropriate tubes for predilution and testing of samples before proceeding.

Sample dilutions are prepared in pyrogen free tubes or bottles. Diluting samples to the routine test dilution is recorded in Appendix 1K.

- Retrieve the required samples from cold room to equilibrate to room temperature before use
- Rack and label the appropriate number of dilution tubes required for each sample as set out in the completed Appendix 1K – Kinetic LAL Sample Results Sheet paperwork. If using pyrogen free test tubes, remove from the bottom of the foil packaging ensuring you do not introduce contamination. If using the black lid screw-top bottles or pre-used tubes ensure that they have been depyrogenated.
- Open the LRW vial, resting the stopper upside down in the pyrogen-free base of the plate seal.
- Dispense LRW into tubes/bottles as set out in the completed Appendix paperwork
- **Ensure that the appropriate PPC spikes have been added to wells as per the plate layout**

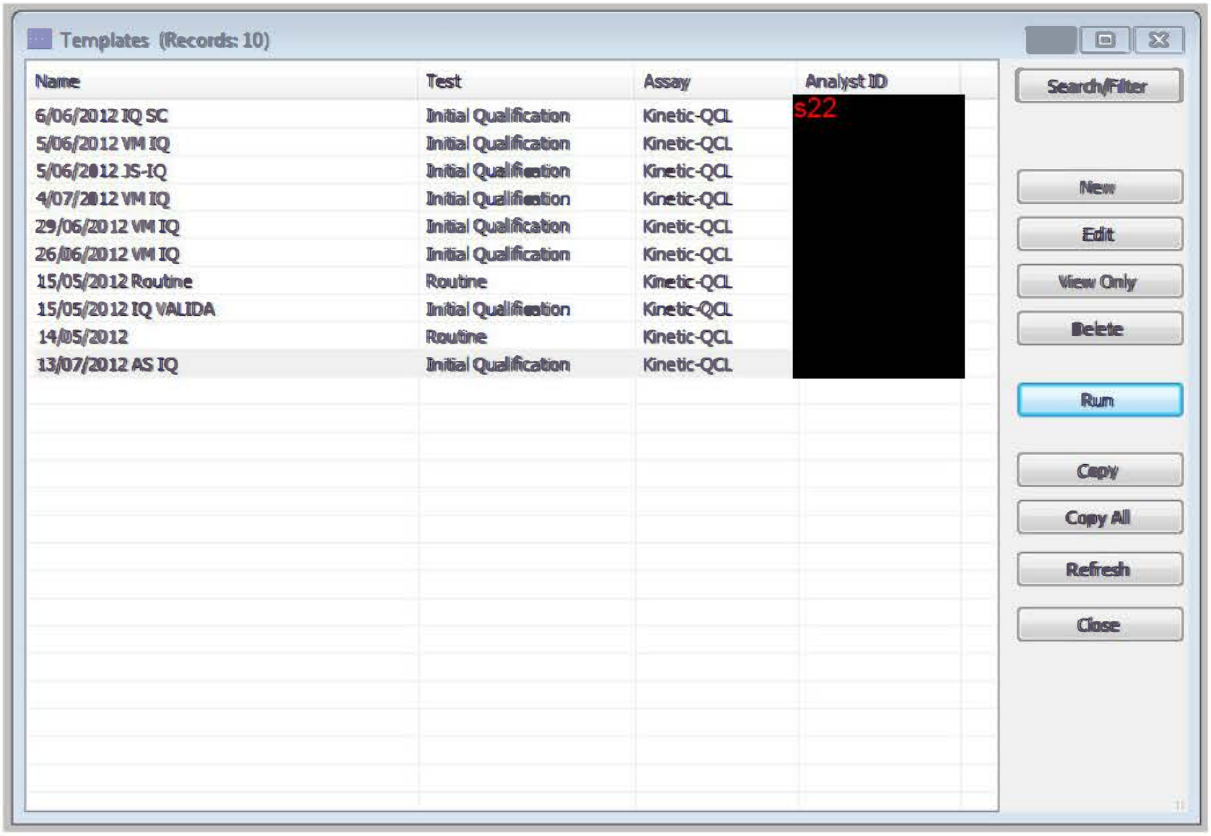
Sample Type
For testing most samples refer to the blue routine samples section here. s22 [redacted]
s22 [redacted]

Routine Samples
- Remove the label from the first sample and place it on the corresponding Sample Results Sheet
- Vortex the first sample on full speed for 10-20 seconds
- Transfer sample from the syringe/vial to the appropriate tube/bottle labelled "Neat"
- Prepare sample dilutions as set out in Appendix 1K, vortexing for 5-10 seconds before transferring to the next dilution tube. Repeat until all dilutions of the first sample are prepared.
- Dispense 100 µl of the final dilution into the 4 appropriate wells as per the plate layout
- Repeat with remaining samples

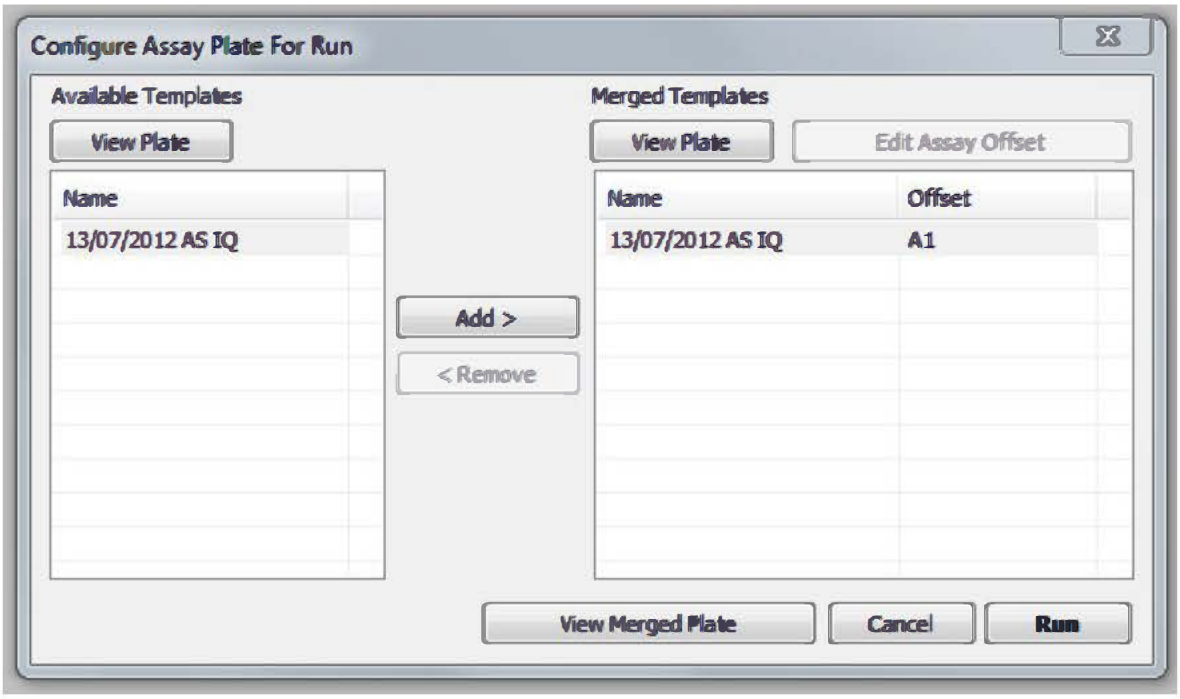
s22 [redacted]

Starting the Assay
Once all samples have been added to the plate, it is then ready for the reaction. Prepare the software for the reaction as follows:

Return to "Templates" screen

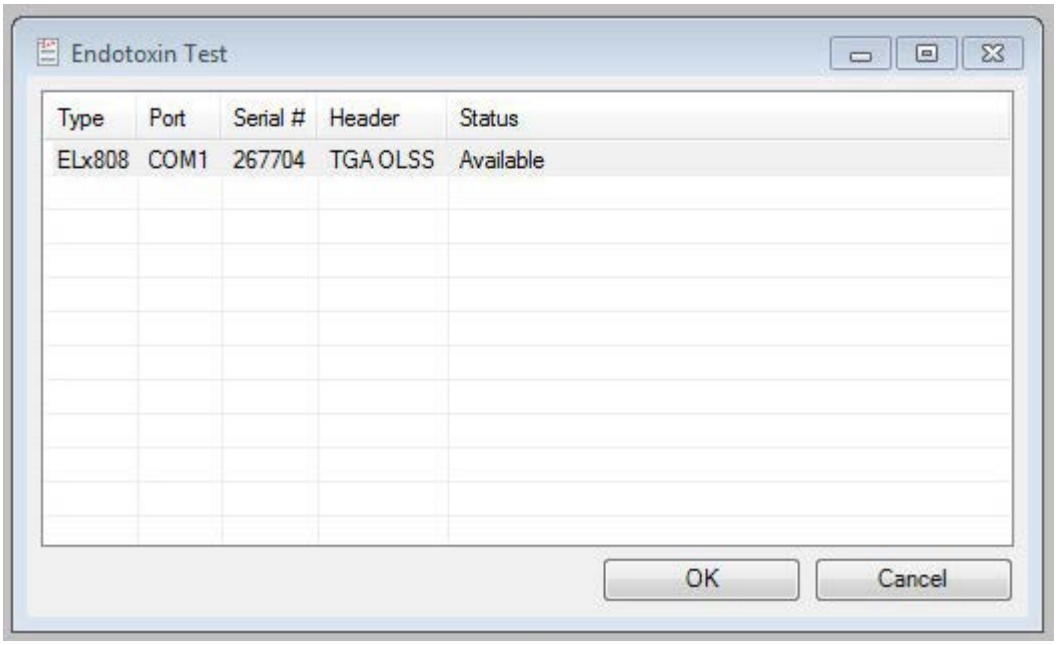


Click on the correct Available Template (prepared earlier) and Add it to the Merged Template field. Select it in this field and click "Run"

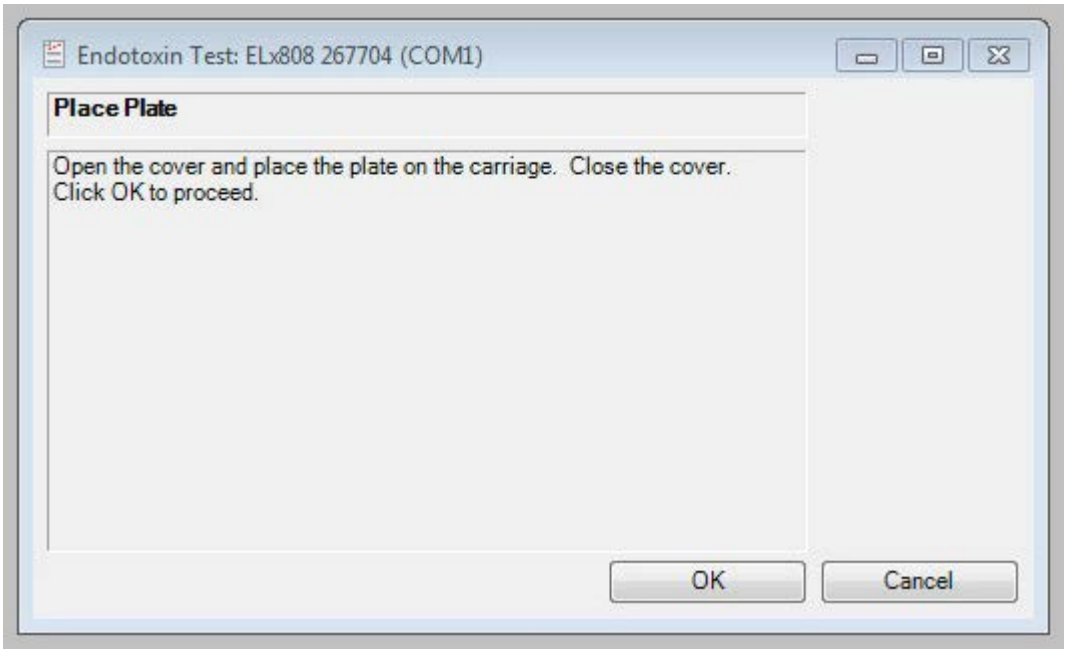


WinKQCL will then ask which plate reader you wish to use. Only one is connected. The Status should read available. Select the ELx808 and click OK.

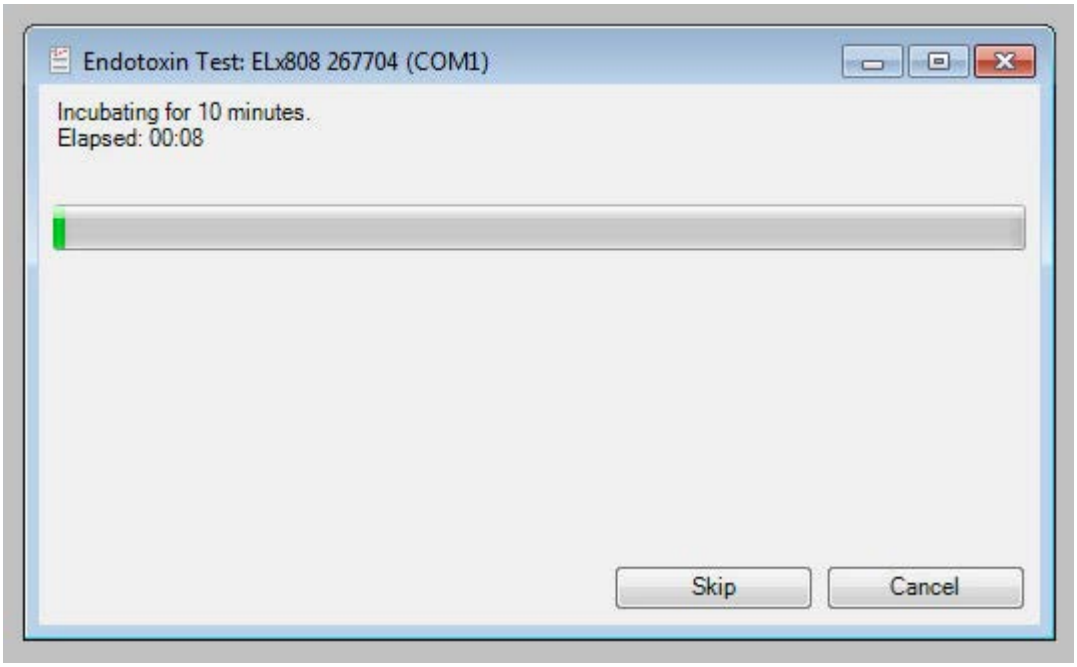
If the ELx808 is "Unavailable", please quit the software and then start the program again. This should reset the connection between the computer and the plate reader. Repeat the steps above.



The plate is then ready for the pre-warming step. Leaving the lid of the plate on, place the plate into the plate reader and click OK



The plate incubates for a minimum of 10 minutes



- Carefully move the mouse cursor AWAY from the “Skip” button. It is not recommended that the pre-warming step is “skipped”. During this incubation ensure that the lysate vial/s and the LAL Reagent Water are equilibrated to room temperature.
- While you are waiting for the pre-warm step to complete, read the remainder of this method again, to ensure you know how the rest of the assay is expected to proceed
- The plate reader will maintain the temperature after the 10 minutes has passed so there is no need to rush. Ensure that the reagent reservoir bag is close at hand.
- Prepare the 8 channel pipettor, ready to dispense 100 µl. Ensure there are sufficient yellow tips for the procedure and they are arranged in the rack in an appropriate configuration for the addition of lysate to the plate (for the IQ assay, this would be one set of 6 and one set of 2 tips). For a routine assay, it would depend on how many samples are required. Ensure that you have plenty of spare tips in case they are required
- It is useful at this point to think about the plate format, how many rows and columns the lysate will be dispensed into, and confirm how many vials of lysate will be required, remembering that each vial can be used for one quarter of a reaction plate (24 wells).

Reconstitution of LAL Reagent

The lysate should be reconstituted according to the manufacturer’s instructions. Currently, using Lonza’s KQCL kit, this involves reconstitution with 2.6 ml of LAL Reagent Water (LRW). This should only be done immediately before it is required for use. Preparation of the LAL reagent should be recorded on a printed copy of Appendix 3K.

- When the plate reader has completed the pre-warm, carefully loosen the rubber stopper/s on the lysate vial/s to allow the vacuum to be released without contaminating the vial and without losing the vial contents.
- Open the lysate vial/s, resting the stopper upside down in the plastic base of the plate seal.
- Open the LRW vial, resting the stopper upside down in the plastic base of the plate seal.
- Reconstitute the lysate with the specified volume of LRW stated on the vial (2.6 ml).
- Replace the stopper on the LRW vial.
- Replace the stopper on the lysate vial and very gently swirl the vial of reconstituted lysate to promote dissolution. Avoid frothing. Repeat with the appropriate number of lysate vials.

- While the lysate is dissolving, carefully remove a reagent reservoir and reseal the bag to maintain pyrogen-free environment.

It is very important to be quick and precise when adding the lysate to the plate, and to avoid bubbles and frothing. The lysate can be dispensed using normal or “reverse” pipetting. Most bubbles will disappear after about 10 seconds.

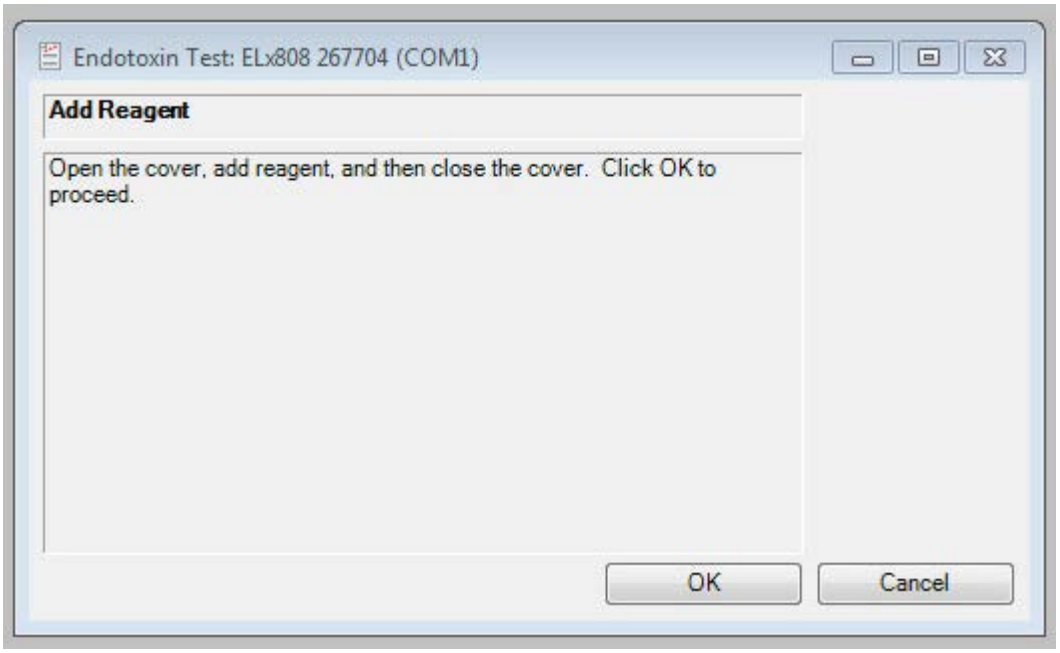
- Move the tip discard close to the plate reader and ensure the 8 channel pipettor (and the single channel pipettor) is set at 100 µl,
- **Only when you are ready**, pour the contents of the lysate vial/s into the reagent reservoir, adding the remaining 100-200 µl from the vial/s using the single channel pipettor
- Move the reagent reservoir with lysate next to the plate reader and position comfortably.
- **Note :** When adding the last column of lysate to the plate there is usually not sufficient lysate in the reservoir to continue using 6 or 8 channels, so it is best to use 2 or 4 tips close to the end.
- Add lysate to plate following the most appropriate guide below

Initial Qualification assay - 4 sets of 6 wells, 1 lysate vial.

- Engage 6 tips onto the multichannel pipettor and perform pre-wet. Open the cover of the plate reader, and perform the following with the plate in the reader.
- Using the same set of tips, and touching just the very top left hand sides of the wells (or nothing at all), pipette 100 µl into the first column of the plate. Using the same set of tips, repeat with the second and third columns of the plate. Either remove 4 tips, or discard all 6 tips and quickly engage 2 tips and quickly pre-wet. Dispense 100 µl into wells A4 & B4. Using the same set of tips pipette 100 µl into wells C4 & D4, and then E4 & F4.

Routine/s22 - 2 or more sets of 8 wells, 1 or 2 lysate vials

- Engage 8 tips onto the multichannel pipettor and perform pre-wet. Open the cover of the plate reader, and perform the following with the plate in the reader.
- Using the same set of tips, and touching just the very top left hand sides of the wells (or nothing at all), pipette 100 µl into the first column of the plate. Using the same set of tips, continue with the subsequent columns of the plate. If the number of samples tested means that all of the lysate will be used, the final column should be pipetted using two tips at a time.



Close the cover on the plate reader and click OK to start the run. The reader will shake the plate for 30 seconds and then perform a reading every 150 seconds until it has reached 40 reads.
Do not open the cover of the reader until the assay is complete otherwise the assay will be invalid.

Assay Acceptance Criteria

The standard curve must meet the following parameters for the assay to be considered valid.

- Correlation coefficient (r) absolute value ≥ 0.980
- Slope between -0.400 and -0.100
- Y intercept between 2.500 and 3.500
- Mean reaction times of blank ≥ mean reaction times of lowest standard
- Coefficient of variation (CV) values for all standards are < 10%

Conclusions

- Prior to printing the computer generated report, the Operator should electronically sign the report and record any deviations from the method during the e-signature procedure
- The results should be transcribed to the Assay Worksheet and Sample Result Sheet/s i.e. % CV's, acceptance criteria parameters, EU/ml, and % PPC Recovery
- Another operator, preferably the person responsible for endotoxin testing, should then record any explanation of unexpected outcomes and sign electronically as a "Reviewer"

The results are stored as part of the assay in the WinKQCL software. Printed versions are stored in Endotoxin Testing Results folders in FC33.

Australian Government
Department of Health and Ageing
Therapeutic Goods Administration

TGA Office of Laboratories and Scientific Services

Operations	Immunobiology / Lot Release
Procedure	Lot release Manual / Protocol Checklist / Gardasil
Written	s22 [redacted]
Authorised	s22 [redacted]
Date Issued	27 October 2011
Revision #	6

Protocol Check List for Gardasil - MSD

(Quadrivalent Human Papillomavirus Types 6, 11, 16, 18 Recombinant Vaccine)

The protocol checklist is to ensure the company has performed all tests as approved in the official application and that these tests comply with the limits set by the company as specified in the European pharmacopoeia monographs.

Protocol / Certificate of Analysis details (WinLIMS *Reason for Test* **[PROTCHECK]**)

Product Number: (circle one)	**Liquid Vial** (0.5ml dose)	**207386**	**TGA Protocol Number:**	
	Pre-filled Syringe (0.5ml dose)	**207388**	**Date Protocol Received:**	
Quadrivalent bulk Lot No.: (eg 2113508)			**Manufacture Date:**	
US Lot No. or Fill Lot No.: (eg 0444F or NK01590)			**Expiry Date** (3 years @ 2-8°C)	
Released by local NRA: (date and agency)		**Yes / No**	**Protocol Evaluated By:** (date)	
Protocol information: **[WINLIMS PARAMETER]**		Satisfactory Not satisfactory	**WinLIMS Approved By:** **Date:**	

Sample details (WinLIMS *Reason for Test* **[BATCHRELCHECK]** or **[BATCHRELMON]**)

Packing Lot Number: (eg NM25110)		**TGA Sample Number:**	
Store No. Or Purchase Order Number:		**Quantity Received:** (doses)	(1x or 10x)
eBS Product Number:		**Date Received:**	
Shipping Conditions		**Market Quantity:** [WinLIMS Parameter]	doses
Manufacturer to Australia: [WinLIMS Parameter]	OK / Not OK	**Sample Appearance:** [WinLIMS Parameter]	OK / Not OK
Cold Chain Data – Min [WinLIMS Parameter]	°C	**Sample Labelling:** [WinLIMS Parameter]	OK / Not OK
Cold Chain Data – Max [WinLIMS Parameter]	°C	**Sample Packaging:** [WinLIMS Parameter]	OK / Not OK
Company to OLSS Ice bricks, Temperature monitors, Freeze-watch indicator	OK / Not OK	**Recommend Release:**	Yes / No
		Sample Evaluator *(date)*	
Sample approved in WinLIMS by *(date)*			

Check that all specifications are met below:

Tests on Monovalent Bulk Adsorbed Product (MBAP) Type 6 Lot No.:		
Working Seed Lot No(s).		
Fermentation Lot No(s).		
Tests	**Specifications**	**Results** (Circle one)
Host Strain Identity (Replica Plating) *A strain of Saccharomyces cerevisiae, designated as* s47	Conforms	PASS / FAIL
Final Aqueous Product (FAP) Lot No.		
Protein Concentration (BCA Assay)	s47	..... µg/ml PASS / FAIL
Purity (SDS Page)	s47	..... % PASS / FAIL
Intact Monomer(SDS Page)	s47	..... % PASS / FAIL
MBAP Lot No.		
IVRP (Enzyme Immunoassay)	s47	..... units/ml PASS / FAIL
Identity (Enzyme Immunoassay)	Conforms (all types)	PASS / FAIL
Sterility – (14 Days) (Membrane Filtration)	Thioglycollate @ 30-35ºC Soybean Casein Digest @ 20-25ºC	PASS / FAIL PASS / FAIL

Tests on Monovalent Bulk Adsorbed Product (MBAP) Type 11 Lot No.:		
Working Seed Lot No(s).		
Fermentation Lot No(s).		
Tests	**Specifications**	**Results** (Circle one)
Host Strain Identity (Replica Plating) *A strain of Saccharomyces cerevisiae, designated as* s47	Conforms	PASS / FAIL
Final Aqueous Product (FAP) Lot No.		
Protein Concentration (BCA Assay)	s47	..... µg/ml PASS / FAIL
Purity (SDS Page)	s47	..... % PASS / FAIL
Intact Monomer(SDS Page)	s47	..... % PASS / FAIL
MBAP Lot No.		
IVRP (Enzyme Immunoassay)	s47	..... units/ml PASS / FAIL
Identity (Enzyme Immunoassay)	Conforms (all types)	PASS / FAIL
Sterility – (14 Days) (Membrane Filtration)	Thioglycollate @ 30-35ºC Soybean Casein Digest @ 20-25ºC	PASS / FAIL PASS / FAIL

Tests on Monovalent Bulk Adsorbed Product (MBAP) Type 16 Lot No.:		.....
Working Seed Lot No(s).		
Fermentation Lot No(s).		
Tests	**Specifications**	**Results** (Circle one)
Host Strain Identity (Replica Plating) *A strain of Saccharomyces cerevisiae, designated as* s47	Conforms	PASS / FAIL
Final Aqueous Product (FAP) Lot No.		
Protein Concentration (BCA Assay)	s47	..... µg/mL PASS / FAIL
Purity (SDS Page)	s47	..... % PASS / FAIL
Intact Monomer(SDS Page)	s47	..... % PASS / FAIL
MBAP Lot No.		
IVRP (Enzyme Immunoassay)	s47	..... units/ml PASS / FAIL
Identity (Enzyme Immunoassay)	Conforms (all types)	PASS / FAIL
Sterility – (14 Days) (Membrane Filtration)	Thioglycollate @ 30-35°C Soybean Casein Digest @ 20-25°C	PASS / FAIL PASS / FAIL

Tests on Monovalent Bulk Adsorbed Product (MBAP) Type 18 Lot No.:		.....
Working Seed Lot No(s).		
Fermentation Lot No(s).		
Tests	**Specifications**	**Results** (Circle one)
Host Strain Identity (Replica Plating) *A strain of Saccharomyces cerevisiae, designated as* s47	Conforms	PASS / FAIL
Final Aqueous Product (FAP) Lot No.		
Protein Concentration (BCA Assay)	s47	..... µg/ml PASS / FAIL
Purity (SDS Page)	s47	..... % PASS / FAIL
Intact Monomer(SDS Page)	s47	..... % PASS / FAIL
MBAP Lot No.		
IVRP (Enzyme Immunoassay)	s47	..... units/ml PASS / FAIL
Identity (Enzyme Immunoassay)	Conforms (all types)	PASS / FAIL
Sterility – (14 Days) (Membrane Filtration)	Thioglycollate @ 30-35°C – No growth Soybean Casein Digest @ 20-25°C	PASS / FAIL PASS / FAIL

Tests on Quadrivalent Bulk Adsorbed Product (QBAP) - Types 6, 11, 16, 18		
Lot No.: ……		
Tests	**Specifications**	**Results** (Circle one)
Sterility – (14 Days) (Membrane Filtration)	Thioglycollate @ 30-35ºC – No growth Soybean Casein Digest @ 20-25ºC – No growth	PASS / FAIL PASS / FAIL

Tests on Quadrivalent Final Container Product (QFCP) Vaccine – Type 6, 11, 16, 18			
Lot No.: ……			
Date of Fill: ……			
Date of Expiry: …… (*Expiry date approval up to 3 years*)			
Tests		**Specifications**	**Results** (Circle one)
pH (pH meter)		s47	…… PASS / FAIL
Aluminium (Spectrophotometric)		s47	…… mg/ml PASS / FAIL
Identity (Enzyme Immunoassay)		s47	PASS / FAIL
Endotoxin (LAL)		s47	…… EU/ml PASS /FAIL
Sterility – (14 Days) (Membrane Filtration)		Thioglycollate @ 30-35ºC – No growth Soybean Casein Digest @ 20-25ºC – No growth	PASS / FAIL PASS / FAIL
Recoverable Volume		At least 0.5 ml (*product no.* s47 *- syringe Only*)	…… PASS / FAIL or N/A
Syringeability		No evidence of needle blockage (*product no.* s47 *- syringe Only*)	PASS / FAIL or N/A
IVRP* (Enzyme Immunoassay)	**Type 6**	s47	…… units/ml PASS / FAIL
	Type 11	s47	…… units/ml PASS / FAIL
	Type 16	s47	…… units/ml PASS / FAIL
	Type 18	s47	…… units/ml PASS / FAIL
Total IVRP* (all types)		s47	…… units/ml PASS / FAIL

***WinLIMS Parameters**

Australian Government
Department of Health and Ageing
Therapeutic Goods Administration

TGA Office of Laboratories and Scientific Services

Operations	Immunology
Procedure	Lot release Manual/virology live vaccines summary of product testing & release criteria - Appendix 7.2
Written	s22 [redacted]
Authorised	s22 [redacted]
Date Issued	16 June 2011
Revision #	1

Appendix 7.2 Virology Live/Inactivated Vaccines - Summary of Product Testing & Release Criteria

Product / Vaccine name ARTG No.	WinLIMS product number	Samples required by TGA: Initial	Samples required by TGA: Further	Summary of the current testing program and criteria for market release.
s22 [redacted]				
Gardasil 124408 (vial) 124410 (PFS)	 207386 207388	5	1	**Testing Summary:** Testing under development. **Criteria for market release:** 1. Satisfactory company manufacture and testing protocols. 2. Acceptable *sample packaging, appearance, labelling, market quantity, cold-chain max and minimum, shipping conditions, sample potency ELISA, vaccine protocol check, and protocol potency ELISA.* Release if there are no concerns with 1 & 2 above. Appropriate follow up with the company is required if there are any concerns with results from 1 & 2 above.

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Document 6

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Australian Government
Department of Health and Ageing
Therapeutic Goods Administration

TGA Office of Laboratories and Scientific Services

Operations	Immunobiology / Lot Release Manual
Procedure	Appendix 11: Production of Sedimentation Freeze Test Controls
Written	s22 [redacted]
Authorised	s22 [redacted]
Date Issued	23/11/2011
Revision #	1

Appendix 11: Production of Sedimentation Freeze Test Controls

PURPOSE:

To produce sedimentation freeze test controls for use in ensuring that aluminium adjuvanted vaccines have not been frozen during shipment

BACKGROUND:

When Aluminium adjuvanted vaccines are frozen this causes the immunogenic particles to aggregate into large crystals. These large crystals could reduce vaccine immunogenicity and cause increase injection site adverse reactions.

The large crystals formed in a frozen vaccine sample can be visualised under a microscope and observed due to their shorter sedimentation time compared to an unfrozen vaccine sample.

The sedimentation test for freezing is performed currently on the following vaccines (See Table 1)

Table 1: Vaccines Currently Tested For Effects of Freezing

Vaccine	Commercial name (Manufacturer)
s22 [redacted]	
Human Papilloma Virus	Gardasil (MSD) s22 [redacted]

RESPONSIBILITY:

It is the responsibility of the relevant Professional Officer to ensure that the sample controls used in the 'Sedimentation Test for Evidence of Freezing' are not expired samples and in current container type.

For each formulation (Dose and Container), Sedimentation test controls are required.

PAPERWORK:

Prior to commencing the procedure obtain and complete the **'Appendix 12: Sedimentation Freeze Test Controls Worksheet'** for each product formulation.

MATERIALS / EQUIPMENT:

Vaccine Samples to be used as controls (3-5 vials/syringes of the same batch)
Permanent Marker Pen or label printer
Timer
Freezer -20°C

METHOD:

1. Select 3-5 vials/syringes of the same batch. Record their details on the 'Sedimentation Freeze Test Control Worksheet'. Record the use of samples in the Sample Receipt Register.
2. Ensure that all vaccine solution is visible in vial/syringe. You may need to remove part of the label.
3. Using a permanent marker pen or pre-printed label, record on each vaccine sample the length of time stored frozen at -20°C, the date and ***Fre*** (abbreviation for freeze). When only 3 vaccines samples available use 0, 1 & 2 hour interval
    a) 0
    b) ½ hr
    c) 1 hr
    d) 2 hr
    e) 4 hr
4. Place samples at -20°C. When freeze storage time is complete, record on worksheet next to freeze time whether the sample was frozen (F) or not frozen (NF).
5. Transfer samples to 2-8°C.
6. Allow freeze treated samples to thaw at 2-8ºC.
7. When all freeze points are complete, shake all time points (0 to 4hrs at -20°C), to ensure particles are suspended.
8. After 5mins of standing record the percentage (%) of sedimentation (volume of clear supernatant)
9. Keep the following samples as the controls:
    (i) non-frozen sample and
    (ii) frozen sample which sediments within 5 minutes.

    If more than one frozen sample sediments within 5 minutes, select the sample which was frozen for the shortest period of time.
10. Sedimentation freeze test controls are stored at 2-8°C in the same fridge as the batch release samples.
11. The completed paper work is stored in the appropriate *Sample Receipt Register* folder in the relevant vaccine section.