

## ASX ANNOUNCEMENT

10 March 2009

### Independent Italian study results confirm potential of Fermiscan Breast Cancer Test in Europe.

**The Italian study results, in summary, indicate sensitivity (ability to accurately detect cancer) of 83% and specificity (ability to accurately detect the absence of cancer) of 76%. These results compare favourably with all Fermiscan Breast Cancer Test results to date.**

Fermiscan today announced the results of an independent Italian study undertaken by an Italian National Health Service Unit, ASLto5, under the supervision of the Centre for Oncological Prevention of the Piemonte Region which approved the study.

The Italian National Health Service Unit, ASLto5, runs a major screening programme for breast cancer under the auspices of the Piedmont Centre for Oncological Prevention (CPO) which conducts approximately 250,000 mammograms per year (refer to <http://www.cpo.it>). The population of Piedmont is approximately 4,000,000 people.

The study followed the format of the Italian screening system. Participants in the study were patients who had a first radiological examination that was read as abnormal by two radiologists. The patients were then recalled for second examination (Mammography, ultrasound, clinical examination, biopsy) and hair from patients willing to participate in the trial was taken and sent to Fermiscan for analysis. The trial consisted of 123 participants.

A copy of the report from the study was provided on request to the President of the Health Committee of the Senate (Parliamentary Health Committee) in Rome. A translated copy of the report is attached to this announcement.

The attached report states;

*“Finally, from the preliminary results there emerges also the possibility of a new paradigm in screening whereby the diffraction test can be used as a procedure for screening at I° level more extensively within a target population of women in the category, using it as a broader instrument of “triage” for women to be sent to II° level. We should bear in mind also that it appears to recognize systemic change and therefore enables management of the disease at an even earlier stage”*

The report is encouraging and concludes with the intention to further evaluate the Fermiscan Breast Cancer Test in a multi-centre study in Europe. The report highlights a comparison of the Fermiscan Breast Cancer Test with other studies of various types of screening tests and an extract of this comparison table is below.

The Italian National Health Service Unit, ASLto5 are currently moving to a European multi centric Fermiscan Breast Cancer Test trial to confirm the relevance of this screening method in a European context.

**RISULTATI PRELIMINARI – IT 123 casi –  
confronto con altre modalità di screening primario**

**Sensibilità = 83%**  
**Specificità = 76%**  
**PPV = 59%**  
**NPV = 92%**

<b>Test</b>	<b>Fonte</b>	<b>Sensibilità</b>	<b>Specificità</b>
<b>PAP test</b>	ACCP 1999	51%	98%
<b>PSA</b>	BMC-FP 2002	86%	33%
<b>FOBT</b>	AJGE 2008	69%	73%
<b>Mx - USA</b>	JAMA 2006	75%	92%
<b>Mx – EU</b>	Linee Guida (Mid Point)	65%	60% <sup>68</sup>

David Young, Fermiscan’s Managing Director said “Fermiscan is grateful for the support we are receiving in Italy from all concerned and we look forward to continuing our progress in Europe”.

For further information, please contact  
 David Young  
 Managing Director  
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## **About Fermiscan**

Fermiscan's principal activity is the commercialisation of an innovative non-invasive diagnostic test for the detection of breast cancer.

The Fermiscan Breast Cancer Test is based on the discovery by an Australian scientist that a change can be detected in the molecular structure of hair from women with breast cancer and this change can be identified by using diffraction of X-rays generated in a synchrotron. The greatest application for the test is in women under 70 years of age and in particular for younger women, where mammography is generally unsuitable.

Fermiscan acquired Sydney Breast Clinic in June 2008.

Fermiscan was named 'Australian Innovator of the year' at the Austrade sponsored annual 'Innovation Day Shoot Out' competition in New York in January 2008.

Fermiscan announced earlier today that it has entered into an Option Agreement granting Hitachi Chemical Company Limited an exclusive license on exercise of the option to establish and operate the Fermiscan Breast Cancer Test in Japan and Korea. The Agreement requires the completion of a feasibility study by Hitachi Chemical over the coming six months and, subject to exercising that option, Fermiscan and Hitachi Chemical will enter into negotiations for licensing the Fermiscan Breast Cancer Test in Japan and Korea.

Fermiscan is a member of the Australian Technology Showcase, a national program aimed at promoting and developing export markets for innovative technology based products. Fermiscan has received support from the NSW Department of State and Regional Development through its various programs such as BioBusiness and BioFirst.

Visit Fermiscan's website at [www.fermiscan.com.au](http://www.fermiscan.com.au)

Visit Sydney Breast Clinic's website at [www.sydneybreastclinic.com.au](http://www.sydneybreastclinic.com.au)



The Hon. Antonio Tomassini.  
President  
Hygiene and Health Committee  
The Senate of the Republic  
**Rome**

p.c.Dott. Antonio Ponti  
Mammographic Screening Director  
CPO Piedmont  
Via San Francesco da Paola 31  
10123 Torino

Ill.mo Hon Antonio Tomassini,

Following your request we attach some short notes on the scientific rationale of the diffraction test on human hair for detection of breast cancer.

The research project undertaken in our ASLto5 is being carried out under the supervision of the Centre for Oncological Prevention (CPO) of the Piemonte Region which has approved the attached study protocol.

We remain at your disposal for any clarification

La Ringraziamo e cogliamo l'occasione per distintamente salutarLa.

Managing Director ASLto5

Dr F. Caruso

Director of the study.

Coordinator of Screening Programme ASLto5

Dr S. Polizzi

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-TRANSLATION FROM ORIGINAL 3-3-2009 ASLto5 -

THE USE OF SYNCHROTRON DIFFRACTION OF HAIR FOR THE DETECTION OF  
BREAST PATHOLOGIES



## THE USE OF SYNCHROTRON DIFFRACTION OF HAIR FOR THE DETECTION OF BREAST PATHOLOGIES

- For about the last ten years X ray diffraction of the hair has been developing as a practical technique for detection of cancer of the breast.
- Numerous studies have looked at this problem, and below we report the rational and biological basis of this test, as well as the results of international studies and an ongoing Italian clinical trial.
- The analysis in these studies document the diffraction test and show that they produce results that are as good as, or better than those required by the European Guidelines.
- In the final section are reported an economic analysis to compare the traditional screening using Mammographic film , with that of the diffraction test of the hair as an instrument of “triage” for asymptomatic women prior to sending them to II level for traditional mammography, ultrasound, MRI and biopsy.*

### *1. Rationale and the current scientific state*

In 1999 an Australian researcher, working in Japan, discovered the presence of a modified intermolecular structure in hairs which are placed in diffraction from X rays generated by a Synchrotron in women diagnosed with breast cancer. The results were published in *Nature* (James Nature 1999;398:33-34).

From the point of view of detection, the diffraction pattern of the hair in women with breast cancer differs from that of healthy subjects in the appearance of a characteristic “ring” which corresponds to a molecular spacing of  $4.44 \pm 0.06$  nm which correlates to an assembly of fibrils of alfa keratin, structured repetitively, but with a random orientation. This superimposes itself on a pattern of signals associated with the packing of the fibrils of the hair and on the links which form at a molecular level between the fibrils. This is illustrated in Figure 1 taken from the original work of Prof. James

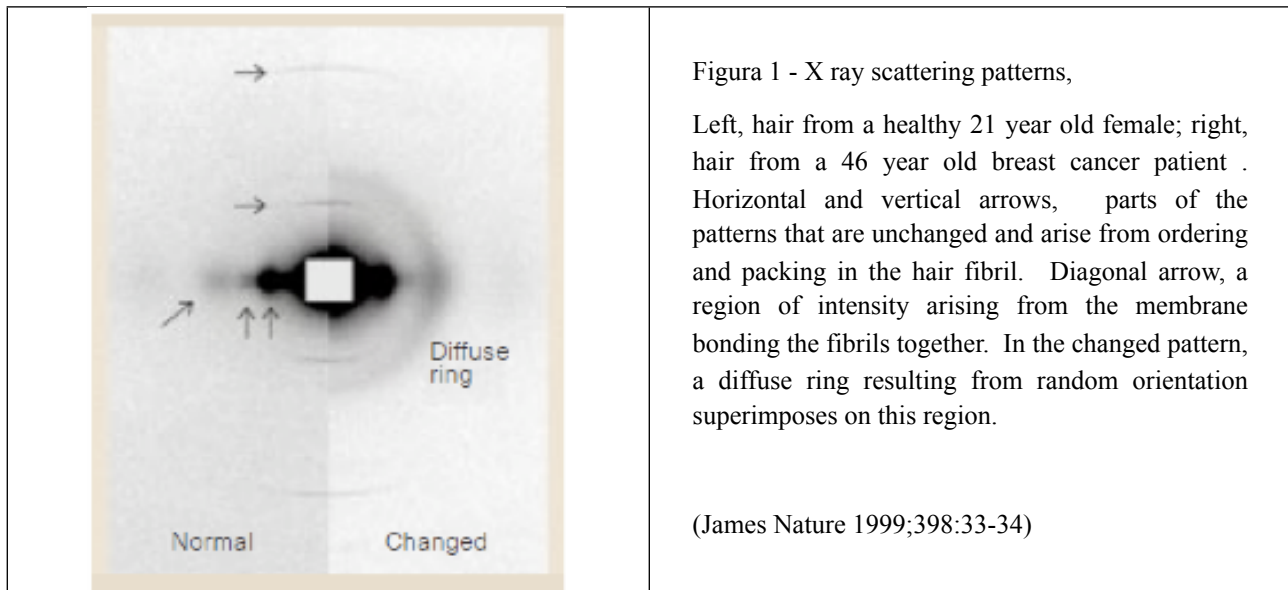


Figura 1 - X ray scattering patterns,

Left, hair from a healthy 21 year old female; right, hair from a 46 year old breast cancer patient . Horizontal and vertical arrows, parts of the patterns that are unchanged and arise from ordering and packing in the hair fibril. Diagonal arrow, a region of intensity arising from the membrane bonding the fibrils together. In the changed pattern, a diffuse ring resulting from random orientation superimposes on this region.

(James Nature 1999;398:33-34)

The original study conducted on 56 cases, showed the ring in 100% of women with known diagnosis of breast cancer. (23 cases of 23) and in 15% of healthy women without genetic risk (3 of 20); in women with genetic risk, 48% (6 of 13) showed the characteristic ring, although they did not have a clinical or radiological diagnosis of breast neoplasia.

As happens often with the use of new methods of diagnosis, initially the results of Prof. James were not confirmed by other researchers. (Briki Nature 1999; 400:226-227, Amenitsch Synchrotron Radiat News 1999;12: 32–4, Mayer J Natl Cancer Inst 2000;92:1092–3).

The original work of James underlined the importance in preparation of the samples, and the necessity of excluding subjects whose hair had been treated with perms or dyes.

In addition, the cases examined in these first studies referred to women after chemo, radio, or endocrine therapy leading to possible confusion.

It is worth observing that these studies were conditioned in their results by:

- A limited number of subjects (10 subjects probably healthy, 7 women and 3 males, and 10 women with breast cancer in the work of Briki in 1999, 9 women with breast cancer and 7 controls in the work of Mayer in 2000) and with a statistical power that is insignificant.
- Methods of sampling that were different, (e.g. pubic hair, body hair, no indication of treatment, analysis of a single hair or clumps of hair etc.);
- Methods of diffraction analysis that were different, or instruments that were different. For example the power of the beams used, in particular the study of Briki conducted at the Synchrotron LURE in Paris and that of Trieste in the work of Amenitsch.

In fact Mayer in 2001, published in collaboration with James, the results of a second study conducted on 7 subjects with breast cancer which, except in one case had been treated with chemo



or hormone therapy and concluded that “*the results demonstrate a clear association between a change in the diffraction pattern of the hair and breast cancer*” (Mayer . J Natl Cancer Inst 2001;93: 873-875).

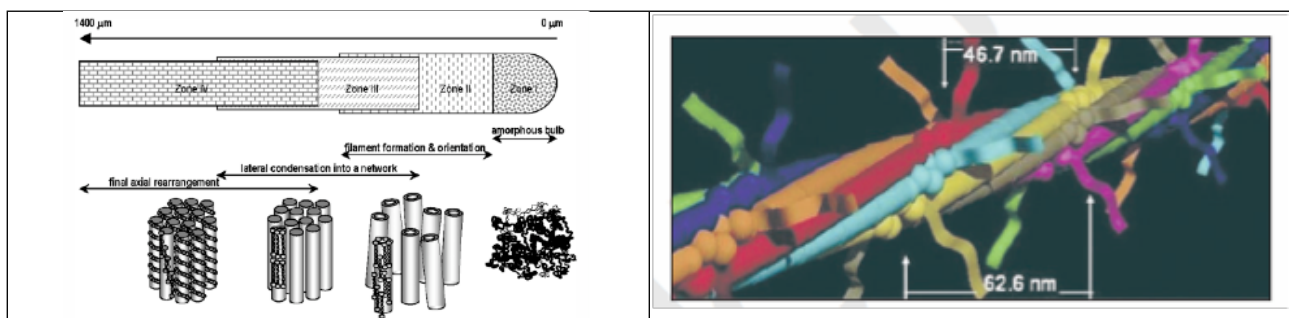
Prof. James, in reply to the criticism by several research groups (James Phys Med Biol 2003;48:137-141), who were unable to replicate the results, explains these results underlining that the majority had “not even been able to obtain a basic diffraction pattern for the hair” (Amenitsch 1999, Howell 2000 [Howell A, Grossmann JG, Cheung KC, Kanbi L, Evans DG, Hasnain SS. Can hair be used to screen for breast cancer? [letter]. J Med Genet 2000;37:297–8] , Mayer 2001)

Of the others, Lawson et al (Lawson J Clin Pathol 2007;60:180-184), studying the histological link between the breast and the skin and hair has demonstrated that:

- Cancer of the breast has systemic effects in changes both of the skin and hair, as for example oestrogen receptors and p53;
- HER2 receptors are expressed both in tumours and in the skin of some subjects, but never expressed in normal subjects;
- Breast cancer can have a systemic effect on the skin and hair and that some cytokines, produced from metastatic tumours, can alter the physiology of the connective tissue of the skin, hair, and other organs

thus documenting a biological basis for the evidence of anomalies in the diffraction pattern of the hair with breast cancer.

Molecular diffraction studies, conducted by the French group Rafik, Briki, Doucet (Rafik J Struct Biol 2006;54 79–88) have demonstrated that hard alpha keratin of the hair are assembled in an axial conformation with links through the disulphide bonding of the cysteine at a distance of 1400 micron from the hair follicle with a characteristic molecular pattern that is visible with the SAXS method (small angle x ray scattering) in a distance that is 4,5 nm in radius from the equatorial zone of the diffraction pattern of the hair (see below). This demonstrates the fact that the analysis of the hair at a distance of 1 cm from the follicle is an area that is suitable for diffraction analysis.



According to the hypothesis of Prof. James, the appearance of a ring would be correlated to the molecular organisation of the alpha keratin, and in particular to the repetition of the diffraction image





of the “tails” of the intermediate filaments which are repeated with constant distance of 46.7 nm (James Med Sci Mon 2003; 9(8):79-83), as shown in the previous page.

## **2. Results of international trials**

At a distance of 10 years from the first observations, the research group of G. Corino e V. James, published the results of various studies conducted on 503 women with and without diagnosis of breast cancer (James & Corino Int J Cancer 2005;114:969-972). The study conducted in Europe, Australia, and North America documented a sensitivity of 100% on 210 women with breast cancer and a specificity which varies from 76% to 80% (according to geography) with 47 false positive cases in 293 women which are presumed to be without breast pathologies on the basis of mammographic screening. Of these 47 cases, 6 had a high genetic risk and 10 had a weak ring on the diffraction image indicating possible breast cancer. If these 16 cases were excluded from the statistical analysis, the specificity would be 89%.

In 2008 the group of Corino and French of Fermiscan Limited (the proprietor of the test) published the results relating to a study of 39 cases of breast neoplasia (Corino Int J Cancer 2008;122:847-956), indicating the method of sampling and analysis on the synchrotron with a sensitivity of 97% and a specificity of 95% in detecting and classifying the cases analysed.

Again in 2008 Fermiscan Ltd conducted a trial in Australia on (inter alia) 721 subjects presenting to a clinic for screening for detection of breast neoplasia.

Of the 721 subjects, 15 had a positive diagnosis on biopsy, and 706 were negative to mammography.

The diffraction images from the synchrotron detected correctly 14 cases of 15 with one false negative on a women of 76 aa (sensitivity 93 % specificity 87 %). Of the remaining 706 negative cases at mammography, the diffraction test was positive for 95 cases and negative for 611 cases with a “false positive” rate of of 13%.

## **3. Italian clinical trial**

In Italy there are several programmes for organised screening for the cervix, the breast and the colon.

ASLto5 of the Piedmont Region – Department 2 of secondary oncological prevention, organises in its own area of around 300,000, a screening programme for breast cancer under the auspices of the Piedmont Centre for Oncological Prevention. (CPO)

The screening is aimed at women starting at 49 aa with a bi annual programme of letters for women from 50 to 69 aa who are given mammographic examination on I° level. The patients with a radiological examination that is dubious or highly suspect according to a letter made by 2





radiologists, are invited to attend an examination to level II°. (mammography, ultrasound, clinical examination, biopsy) to confirm or not as early as possible the suspicion of breast neoplasia in order to treat the disease.

After having studied the literature above, ASLto5, contacted the researchers of Fermiscan Ltd, (the proprietor of the test) and became involved in a clinical trial of the diffraction test of the hair, selecting as study population, women invited to II level. The trial has been authorised by the health authorities and the CPO *taking into account the simplicity and non invasiveness of the test that requires simply the collection of about 20 hairs from close to the skin.*

To date 123 cases at II° level have been analysed representing a population base of around 3,500. Of these 36 had a cyto-histology showing breast tumours or lesions at high risk. (e.g. Ductal Hyperplasia Atypica). Of the 36 cases, 30 resulted positive to the diffraction test and one case was classified as abnormal as the image shows different characteristics.

Of the 66 cases classified by the radiologists as negative to the mammography-ultrasound-clinical or biopsy examination, 21 cases were positive to the diffraction test and 44 were negative to the test.

Of the 21 false positives, 11 cases had a high genetic risk, and 14 had events that required examination and recall and should have been recalled anyway. Additionally the results are in general confirmed by the Gail Score and Acute Risk Score of each individual patient..

The protocol of the trial is predicated on the basis of a statistical power of 95% and a recruitment of 120 cases at II° level. The final results will be published including an analysis of the test with the clinical information from the national database (SQTM) which manages the collection of data from the national screening programme.

#### **4. Discussion on the analysis of costs and advantages/disadvantages.**

The results are well within the standards of quality proposed by the *European Guidelines for Quality Assurance in Breast Cancer Screening and Diagnosis. (Fourth Edition)* and we can suggest the following observations in the case of adoption of this test at a national level.

- In the context of traditional screening with mammography it is well known that there exists a number of so called “interval cancers” which include a quota of “false negatives” together with small lesions as well as real interval cancers, that is to say cases not visible to mammography and with a growth superior to the biannual screening. In fact the Guidelines quota for interval cancers is 30% of the incidence rate in absence of screening for the first year and 50% for the second. The acceptable average is given as 40%.
  - *With the synchrotron diffraction test, which can be done annually, there is a rate of “false negatives” much lower than those normally present in an organised screening program.*
- The recall rate, according to the European Guidelines should be 7% for the first round and 5% for the second round (with much higher percentages in North America - 11.6%),



- Using this test, the recall rate would be reduced by 45%. That is to say that after first level screening, we would recall only 55% of the current recall rate for second level screening because the test has a much higher Negative Predictive Value than first level mammography. The saving in anxiety for the general population and the ability of existing services to focus on the important cases would be a great benefit.
- Finally, from the preliminary results there emerges also the possibility of a new paradigm in screening whereby the diffraction test can be used as a procedure for screening at I° level more extensively within a target population of women in the category, using it as a broader instrument of “triage” for women to be sent to II° level. We should bear in mind also that it appears that recognises systemic change and therefore enables management of the disease at an even earlier stage.
- The following table shows the results of the trial compared to other screening methods. We are currently moving to a multi centri trial with other regions (Veneto, Liguria) and are in discussion with the Department of Savoy to make it a European multi centric trial to confirm the relevance of this screening method on a European level.

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