

RESEARCH ARTICLE

# Manual vs automated $^{68}\text{Ga}$ -radiolabelling—A comparison of optimized processes

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## Abstract

A critical factor for clinical practice is the production of  $^{68}\text{Ga}$  radiopharmaceuticals manufactured manually or through an automated procedure.  $^{68}\text{Ga}$  radiopharmaceuticals are often prepared manually, although this method can lead to an increased operator's radiation dose and potential variability within production. The present work compares  $^{68}\text{Ga}$ -radiolabelling (PSMA-11; DOTA-TOC) utilizing a cassette module (GAIA; Elysia-Raytest; Germany) with a manual setup for routine clinical production with regard to process reliability and reproducibility.

## KEYWORDS

automation, cassette module, clinical routine, gallium-68, DOTA-TOC, PSMA-11

## 1 | INTRODUCTION

In recent years, the application of  $^{68}\text{Ga}$  radiopharmaceuticals has increased for positron emission tomography (PET) imaging in research as well as in clinical practice. Gallium-68 is available from a  $^{68}\text{Ge}/^{68}\text{Ga}$ -generator because of its convenient nuclear properties. Its radiolabelling potential with cyclic conjugates and its short half-life ( $T_{1/2} = 67.71$  min) qualifies it for PET imaging with probes of short biological half-life.<sup>1</sup>

The rise of gallium-68 started with the development of somatostatin analogue edotreotide (DOTA-TOC), which targets tumours overexpressing somatostatin receptors.<sup>2</sup> Rapid accumulation in neoplastic tissue and fast clearance from healthy organs enables the delivery of a high dose of radiation on the target site and thus preserves the surrounding healthy tissue.<sup>3</sup> DOTA as chelator makes it possible to apply the same molecule for diagnosis and therapy simply by the choice of radionuclide. This so-called theranostic approach, nowadays, well

established with gallium-68/lutetium-177 as diagnostic/therapeutic pair, initiated the growing interest in radiometals for clinical application beyond technetium-99m.

With the introduction of PSMA-11 and PSMA-617 for prostate cancer (PC) theranostics,<sup>4,5</sup> a second boom of the still exotic PET radionuclide gallium-68 started. PC is one of the most common causes of cancer-related mortality in western societies.<sup>6</sup> Prostate-specific membrane antigen (PSMA) is a transmembrane molecule in prostate tissue and highly overexpressed in PC.<sup>7,8</sup> Its extracellular N-terminal part, containing the catalytic domain, is suitable for selective tumour targeting.<sup>9</sup> Due to the low expression of PSMA in healthy tissue, with the exception of salivary and lacrimal glands, high-dose radioligand therapy is possible. As low-molecular-weight compounds presented very promising properties as PC imaging agents,<sup>10–13</sup> urea-based peptidomimetic inhibitors with a high affinity to PSMA were developed. From those potent agents, the DOTA derivative PSMA-617 emerged as a

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powerful theranostic tool for PC. Both compounds, PSMA-11 and PSMA-617 are now well established in clinical practice.

One of the critical factors in clinical practice is the production of a radiopharmaceutical. In many cases, preparation of  $^{68}\text{Ga}$  radiopharmaceuticals is still manual (Figure 1), although this method would not be adequate for routine clinical applications. The two main problems are the radiation dose to the operator to and potential variability within production.

To avoid these problems in clinical routine, two general concepts were established: using (a) synthesis modules and most recently (b) radiolabelling single vial cold kits. Both concepts should guarantee an easy, safe, and reliable production of  $^{68}\text{Ga}$  radiopharmaceuticals with stable high yields and pharmacopoeia compliant product quality.

The radiolabelling kits should make the production of  $^{68}\text{Ga}$  radiopharmaceuticals as easy as the production of  $^{99\text{m}}\text{Tc}$ -radiopharmaceuticals. As stated by the European Pharmacopoeia (Ph Eur) in general chapter “Extemporaneous Preparation of Radiopharmaceuticals” is the marketing authorization holder of a licensed kit responsible to ensure that the kit complies with the requirements of its marketing authorization, while the radiopharmacy using that licensed kit carries the responsibility for the quality of the preparation and the handling. If the instructions for use are not strictly followed or if one or more components used for the preparation do not have marketing authorization, it is the responsibility of the radiopharmacy to demonstrate that the quality of the final preparation is suitable for the intended use.<sup>14</sup>

Consequently, preparation as well as quality control of a licensed kit require at least the equipment according

to the instructions provided by the manufacturer. In addition, minimum contaminated waste materials remain. It has to be noted that this is only true for licensed kits in combination with a licensed generator. In contrast, unlicensed kits or a licensed kit used with an unlicensed generator also require quality control according to the monograph and additionally, local authorities may require more detailed quality control even for licensed kits.

Admittedly, those kits contain relatively high amounts of precursor and additional filler materials, still require manual handling, and are not available for most precursors and applications. Up to now, only cold kits for radiolabelling PSMA-11 (eg, illumet™) and DOTA-TOC (eg, NETSPOT®; TOCSan) are commercially available. In addition, the use of unpurified generator eluates requires very strict specifications for the generators in terms of  $^{68}\text{Ge}$  breakthrough to avoid radionuclidic impurities in the final product. Nevertheless, they are a possibility for small sites to offer  $^{68}\text{Ga}$  radiopharmaceuticals to their patients without great expense.

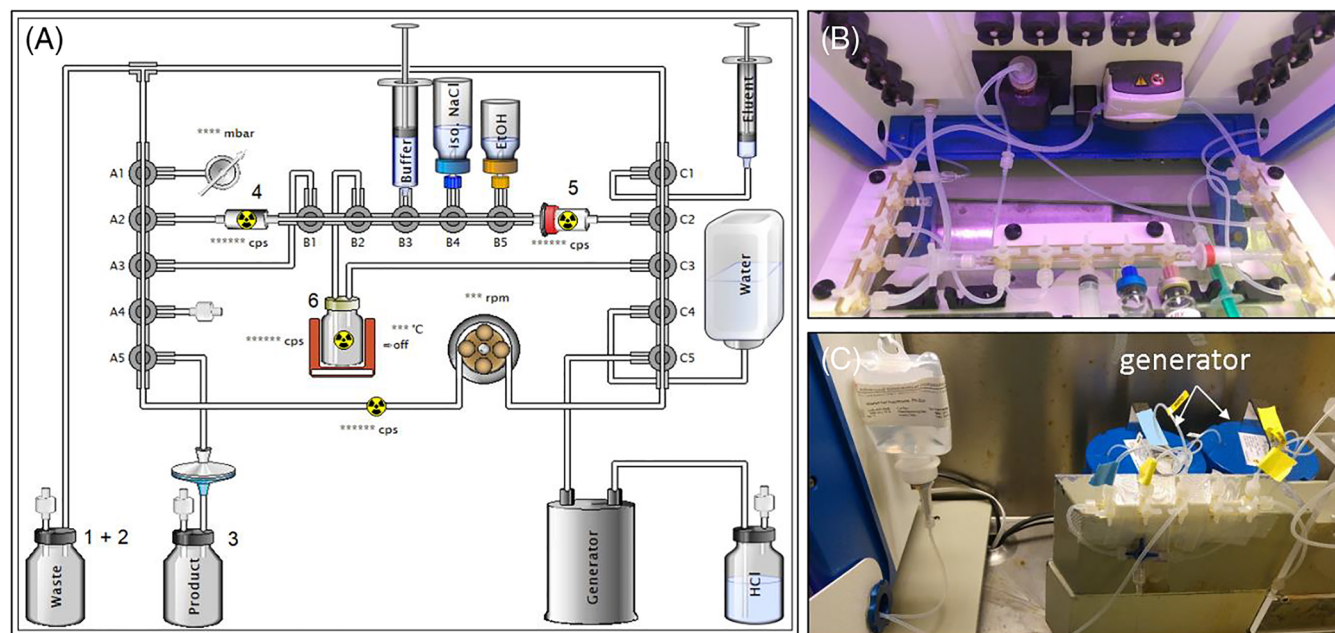
Compared with kit preparations, the synthesis module-based production (Figure 2) requires a fully equipped laboratory and quality control. Starting with the increased use of gallium-68 in nuclear medicine automation of the traditional manual synthesis was promoted. Today, those systems are designed with respect to Good Manufacturing Practice (GMP) Guidelines provided for example by the FDA, EU/EMA, ICH, WHO, or others.<sup>15</sup> They use software and methods designed to minimize user interventions and utilize single-use consumables produced under GMP standards.

Accordingly, the amount of contaminated waste materials is higher because of the procedure as well as the complete quality control. Nevertheless, these systems are suitable for a variety of tracers and in most cases for several radionuclides (eg, Scintomics GRP series, Eckert & Ziegler Modular-Lab PharmTracer, and Trasis AllInOne). Additionally, the module system enhances the production process in terms of higher reliability and reduced variability.<sup>3,16,17</sup>

The present work focuses on the advantages of a commercial automated cassette module for the radiolabelling of bioconjugates with gallium-68 in clinical practice compared with the manual radiolabelling. Both compounds applied (DOTA-TOC and PSMA-11) were produced in our nuclear medicine frequently using a manual method, which was optimized for our site-specific needs. The replacement by a module was indispensable to satisfy the authorities and improve staff radiation safety. This retrospective study compares the site-specific optimized standard manual procedure



**FIGURE 1** Example setup of a manual  $^{68}\text{Ga}$ -radiolabelling for clinical practice



**FIGURE 2** Example for an automated module system used in clinical routine (GAIA, Elysia-Raytest). A, Screenshot of the graphical user interface of module showing the method used. B, Cassette mounted in the open module. C, Module-generator setup in the hot cell

with the standard process given by the manufacturer and a site-specific adjusted.

Performance evaluation of the module used was in terms of AY and RCY. The yields AY and RCY were measured for reliability and reproducibility of the process.

## 2 | MATERIALS AND METHODS

### 2.1 | Automated synthesis

Gallium-68 was obtained from a 1.85-GBq  $^{68}\text{Ge}/^{68}\text{Ga}$  generator (iThemba Labs, South-Africa). Synthesis was performed on the automated cassette module GAIA from Elysia-Raytest (Straubenhardt, Germany), utilizing the standard radiolabelling methods. The detectors of the synthesis module were calibrated with a dose calibrator (ISOMED 2010, MED Nuklear-Medizintechnik Dresden GmbH, Dresden, Germany) as reference. DOTA-TOC, PSMA-11, and standard fluidic and reagent kit (contains all consumables necessary except peptide) for gallium-68 radiolabelling of peptides, all are of GMP grade, were purchased from ABX advanced biochemical compounds (Radeberg, Germany). The SCX included in the reagent kit was replaced by 200-mg Strata SCX (Phenomenex, USA). Optimization of the constitution of the reaction mixture occurred with regard to the different SCX and

optimum pH for the respective precursor. Automated synthesis includes subsequent  $\text{C}_{18}$  purification as well as sterile filtration. Ethanol Ph Eur was purchased from Merck (Darmstadt, Germany).

$44.88 \pm 5.54\text{-}\mu\text{L}$  DOTA-TOC (1 mg/mL) were radio-labelled in  $3.35 \pm 0.19\text{-mL}$  ammonium acetate buffer (0.08M; reagent kit),  $473.13 \pm 24.46\text{-}\mu\text{L}$  eluent (reagent kit), and  $200\text{-}\mu\text{L}$  ethanol with  $1.292 \pm 0.385\text{-GBq}$  gallium-68. After radiolabelling ( $94 \pm 2^\circ\text{C}$ ;  $479.1 \pm 12.2\text{ s}$ ), the reaction mixture (total volume of  $4.07 \pm 0.22\text{ mL}$ ) was diluted with 5-mL water (ethanol concentration  $\sim 2\%$ ). The diluted reaction mixture subsequently passed over a  $\text{C}_{18}$  cartridge and washed with water. The purified product eluted with 1.5-mL 60-vol% ethanol followed by 8.5-mL saline and sterile filtered to obtain the final formulation.

$25.79 \pm 4.27\text{-}\mu\text{L}$  PSMA-11 (0.1 mg/mL) were radio-labelled in  $3.65 \pm 0.16\text{-mL}$  ammonium acetate buffer (0.08M; reagent kit),  $460.19 \pm 19.03\text{-}\mu\text{L}$  eluent (reagent kit), and  $201.69 \pm 22.93\text{-}\mu\text{L}$  ethanol with  $1.289 \pm 0.233\text{-GBq}$  gallium-68. After radiolabelling ( $85 \pm 2^\circ\text{C}$ ;  $310 \pm 9\text{ s}$ ), the reaction mixture (total volume of  $4.44 \pm 0.21\text{ mL}$ ) was diluted with 5-mL water (ethanol concentration  $\sim 2\%$ ). The diluted reaction mixture subsequently passed over a  $\text{C}_{18}$  cartridge and washed with water. The purified product eluted with 1.5-mL 60-vol% ethanol followed by 8.5-mL saline and sterile filtrated to obtain the final formulation.

## 2.2 | Manual synthesis

Gallium-68 was obtained from a 1.85-GBq  $^{68}\text{Ge}/^{68}\text{Ga}$  generator (iThemba Labs, South-Africa). The eluate was postprocessed using ethanol-based postprocessing as previously described.<sup>18</sup> The manual synthesis was carried out in a MHR 13 thermo shaker (Hettich-Benelux, Geldermalsen, Netherlands) at a temperature of 95°C. After addition of the  $^{68}\text{Ga}$  eluate (composition 90-vol% ethanol/10-vol% 0.9 N HCl) to the reaction mixture pH was measured and adjusted if needed; 3M ammonium acetate solution as well as solutions for  $^{68}\text{Ga}$  postprocessing were produced in house.

$55.89 \pm 7.81$   $\mu\text{L}$  DOTA-TOC (1 mg/mL) were radio-labelled in  $0.93 \pm 0.17$ -mL ammonium acetate solution and  $1.02 \pm 0.14$ -mL eluate containing  $1.294 \pm 0.455$ -GBq gallium-68 at a pH of 3.6–3.8. The reaction mixture was diluted with water and passed over a  $\text{C}_{18}$  cartridge. The final product was eluted with 1.5-mL 60-vol% ethanol followed by 8.5-mL saline solution and sterile filtrated into the product vial.

$43.70 \pm 19.30$ - $\mu\text{L}$  PSMA-11 (0.1 mg/mL) were radio-labelled in  $1.00 \pm 0.08$ -mL ammonium acetate solution and 1-mL eluate containing  $1.368 \pm 0.353$ -GBq gallium-68 at a pH of 4 to 4.2. The reaction mixture was diluted with 8-mL saline and sterile filtrated into the final product vial.

## 2.3 | Quality control

For quality control, an aliquot of 50  $\mu\text{L}$  was retained from the final product before measurement of radioactivity. All chemicals were pure or analytical grade and used as received, unless otherwise specified. Radioactivity of the final product was measured with a dose calibrator (ISOMED 2010, MED Nuklear-Medizintechnik Dresden GmbH, Dresden, Germany). Radiochemical purity (RCP) was determined using silica-gel coated aluminium TLC-plates (silica 60 F254.5X4.5 cm Merck, Darmstadt, Germany), as well as glass microfiber chromatography paper impregnated with silica-gel (iTLC-SG, Agilent Technologies, Santa Clara, California) and analysed using a single trace radioTLC-scanner (PET-miniGita, Elysia-Raytest, Straubenhardt, Germany) and evaluation software (Gina Star TLC, Elysia-Raytest, Straubenhardt, Germany). For DOTA-TOC, iTLC-strips were developed in 0.1M citric buffer (pH 4, Merck, Darmstadt, Germany) as well as in 1M ammonium acetate/methanol (1:1), for PSMA-11 a TLC-strip was developed in 0.1M citric buffer (pH 4, Merck, Darmstadt, Germany) and an iTLC-strip in 1M ammonium acetate/methanol (1:1). Additionally, radio-HPLC was used to determine the RCP and identification

of the product species. RadioHPLC was performed utilizing Agilent 1260 Infinity II reverse phase HPLC system (Agilent Technologies, Santa Clara, California) equipped with Gabi  $\gamma$ -HPLC flow detector (Elysia-Raytest, Straubenhardt, Germany) and a PC interface running Gina Star (Elysia-Raytest, Straubenhardt, Germany). A Nucleodur 100-3  $\text{C}_{18}$  ec 125/4 column (Macherey-Nagel GmbH & Co. KG, Düren, Germany) was used. The gradient utilized mobile phase A (deionized water + 0.01% trifluoroacetic acid) and mobile phase B (acetonitrile + 0.01% trifluoroacetic acid). Flow rate was 0.7 mL/min starting with 100% A/0% B to 0% A/100% B within 20 min. Afterwards, gradient parameters change to 50% A/50% B within 5 min; pH was measured using pH-indicator strips MColorpHast 2.0–9.0 (Merck, Darmstadt, Germany). The approximate half-life of gallium-68 was determined using the dose calibrator (ISOMED 2010, MED Nuklear-Medizintechnik Dresden GmbH, Dresden, Germany). The energy of gallium-68 and germanium-68 content were measured using a multichannel analyser for  $\gamma$ -spectroscopy (MUCHA, Elysia-Raytest, Straubenhardt, Germany). Appearance was checked visually. Filter integrity was tested with GAIA (Elysia-Raytest, Straubenhardt, Germany).

## 2.4 | Calculations

RCP was determined by radioTLC and radioHPLC unless otherwise stated.

AY as well as RCY were calculated in two different ways. First, based on the activity trapped on the SCX, activity trapped on  $\text{C}_{18}$ , and remaining activity on  $\text{C}_{18}$  after final formulation, all measured during the process with the detector included in the module. Second, based on the activity of the final product vial, measured using a dose calibrator, and the activity trapped on SCX as determined by the detector included in the module. Unless otherwise stated, AY and RCY presented were calculated with method one.

$t_{\text{process}}$  was calculated based on the time points obtained when measuring the final product activity and the activity trapped on the SCX (module) or measured in the eluate (manual method).  $t_{\text{process}}$  depends on the setup of the module and the time needed to transfer the final product to the dose calibrator and is therefore site specific.

Volume activity ( $A_v$ ) and apparent molar activity were calculated based on activity of the final product measured with the dose calibrator.

Statistics were calculated with PRISM Version 8.0.2. All data (based on the revised data set) are expressed as mean  $\pm$  SD. Groups were compared using the  $t$  test. All



statistical tests are two tailed, with a *P* value of .05 representative for significance.

### 3 | RESULTS

In the present study, reliability and reproducibility of automated radiolabelling were compared with manual radiolabelling. All data obtained from routine clinical production were retrospectively analysed with regard to these questions. Automated radiolabelling was performed using a cassette module system (GAIA, Elysia-Raytest). As the present study focuses on the module performance, module-independent parameters were not discussed in detail.

#### 3.1 | [<sup>68</sup>Ga]Ga-DOTA-TOC

A data set of 306 batch records consisting of 47 manual and 259 automated synthesis was analysed (Table 1).

The mean (*M*) starting activities and corresponding standard deviations (*SD*) are in the same range for both automated and manual synthesis.

There are significant differences between both methods for process duration. Conducting the manual method leads to the final product within an average of  $22:52 \pm 3:47$  minutes compared with  $18:35 \pm 5:53$  minutes with the automated method (Table 1).

The significantly lower yield of  $55.2 \pm 20.2\%$  (AY) for the manual method vs  $78.4 \pm 15.2\%$  (AY) for the automated process reflects the prolonged synthesis duration and process variabilities. Similar results were found for the process duration-independent decay-corrected yields

(RCY),  $69.5 \pm 19.7\%$  for the manual method vs  $89.1 \pm 17.4\%$  for the automated process.

Admittedly, the data set includes data from all batches produced independently from the cause of failed synthesis. This affects the standard deviation and the measures of reliability and reproducibility, as well as the AY, the measure for suitability and RCY, and the measure for performance of the entire process. As the goal was to compare two processes, the data set was analysed again to determine the causes of the particular failed synthesis. A failure is a synthesis producing a final product not fulfilling the product specification (based on the monograph for [<sup>68</sup>Ga]Ga-DOTA-TOC of the European Pharmacopoeia<sup>14</sup>). It did not matter whether further purification was possible or not. Causes of failed synthesis were identified and classified in process-related (eg, malfunctioning solution transfer) and nonprocess-related (eg, low peptide quality). Exclusion of data of nonprocess-related failed synthesis leads to the revised data set.

Overall data of 43 synthesis (13 manual; 30 GAIA) were excluded. This means removal of 14.1% (27.7% manual, 11.6% GAIA) failed syntheses induced by nonprocess-related causes. Non-process-related causes observed were low peptide quality, incorrect or poor preparation of the synthesis by the operator, a damaged generator, and a power failure in the building. There are 0 failed synthesis in the revised data set for both methods.

As shown in Table 1, both processes starting activities and process duration are nearly unaffected ( $22:21 \pm 2:50$  min manual;  $18:19 \pm 5:41$  min GAIA). The mean values for AY and RCY increase, whereas the standard deviation drops from  $55.2 \pm 20.2\%$  to  $62.3 \pm 13.1\%$

**TABLE 1** Comparison of GAIA and manual synthesis data for [<sup>68</sup>Ga]Ga-DOTA-TOC including and excluding nonprocess-related production failures

		Complete Data Set				Revised Data Set			
		GAIA		Manual		GAIA		Manual	
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Process	n	259		47		229		34	
	A <sub>Start</sub> , GBq	1.292	0.385	1.294	0.455	1.304	0.388	1.300	0.257
	A <sub>Product</sub> , GBq	0.915	0.276	0.655	0.277	0.963	0.236	0.714	0.257
	t <sub>Process</sub> , mm:ss	18:35	5:53	22:52	3:47	18:19	5:41	22:21	2:50
	AY, % <sup>a</sup>	78.4	15.2	55.2	20.2	81.0	11.1	62.3	13.1
	RCY, % <sup>b</sup>	89.1	17.4	69.5	19.7	92.2	12.7	74.4	16.3

<sup>a</sup>Nondecay corrected.

<sup>b</sup>Decay corrected.

(AY) and  $69.5 \pm 19.7\%$  to  $74.4 \pm 16.3\%$  (RCY) for the manual and  $78.4 \pm 15.2\%$  to  $81.0 \pm 11.1\%$  (AY) and  $89.1 \pm 17.4\%$  to  $92.2 \pm 12.7\%$  (RCY) for the automated procedure, while synthesis duration remains nearly unaffected.

Within the evaluation period, the automated process was customized with two adjustments to improve radiolabelling. The manual method remained unchanged for all batches performed.

First, substitution of the Agilent SCX cartridge, provided with the kit, by Phenomenex SCX cartridge. Second, additional 5-vol% ethanol in the reaction mixture.

Therefore, the effect of the particular adjustments on the automated process was analysed by pooling and processing the corresponding batch records. Overall, three subgroups were created: first, synthesis utilizing the original Agilent SCX provided with the

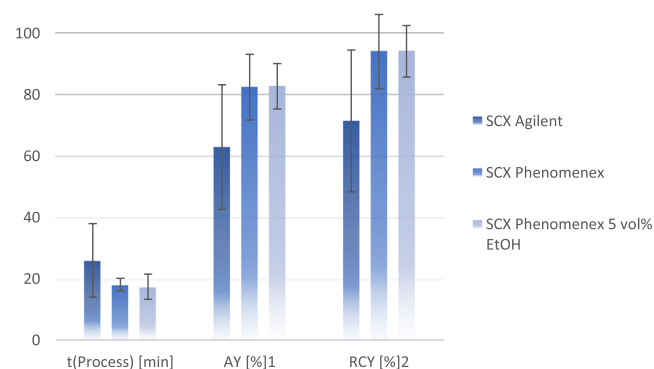
radiolabelling kit, without additional ethanol (20 batches); second, synthesis with the substitute Phenomenex SCX without additional ethanol (28 batches); and third, synthesis with the substitute Phenomenex SCX with 5-vol% ethanol in the reaction mixture (181 batches). All data, depicted in Figure 3, were obtained from the revised data set.

As shown in Figure 3, the effect of the two adjustments on yields as well as reproducibility is significant. The process duration drops from  $26:04 \pm 11:59$  minutes to  $17:29 \pm 4:07$  minutes, which means a reduction of 33%. Simultaneously, AY increases from  $63.0 \pm 20.3\%$  to  $82.8 \pm 7.4\%$  and RCY from  $71.5 \pm 23.1\%$  to  $94.2 \pm 8.4\%$ .

Table 2 compares the final method utilized with GAIA, including the substitute SCX cartridge and 5-vol% ethanol in the reaction mixture (181 batch records), with manual synthesis (34 batch records). The mean starting activities and corresponding standard deviations are in the same range for both automated and manual synthesis.

There were significant differences between both methods for process duration. The manual method leads to the final product within an average of  $22:21 \pm 2:50$  minutes compared with  $17:29 \pm 4:07$  minutes with the optimized automated method (Table 2).

The significantly lower yield of  $62.3 \pm 13.1\%$  (AY) for the manual method opposite to  $82.8 \pm 7.4\%$  (AY) for the automated process reflects the prolonged synthesis duration and process variabilities of the manual method. Decay-corrected yields (RCY) are similar,  $74.4 \pm 16.3\%$  for the manual method vs  $94.2 \pm 8.4\%$  for the automated process.



**FIGURE 3** GAIA synthesis utilizing the original method compared with the two implemented adjustments [ $^{68}\text{Ga}$ ]Ga-DOTA-TOC taking the revised data set as a basis. <sup>1</sup>nondecay corrected; <sup>2</sup>decay corrected

**TABLE 2** Comparison of final method of GAIA synthesis of [ $^{68}\text{Ga}$ ]Ga-DOTA-TOC utilizing Phenomenex SCX and 5-vol% ethanol compared with manual

		Revised Data Set		Additional 5-vol% EtOH			
				SCX Phenomenex		Manual Synthesis	
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Process	n	229		181		34	
	A <sub>Start</sub> , GBq	1.304	0.388	1.318	0.219	1.300	0.257
	A <sub>Product</sub> , GBq	0.963	0.236	1.024	0.191	0.714	0.257
	t <sub>Process</sub> , mm:ss	18:19	5:41	17:29	4:07	22:21	2:50
	AY, % <sup>a</sup>	81.0	11.1	82.8	7.4	62.3	13.1
	RCY, % <sup>b</sup>	92.2	12.7	94.2	8.4	74.4	16.3

Notes. The revised data set was used as basis for this comparison. Data of the complete revised data set are included too.

<sup>a</sup>Nondecay corrected.

<sup>b</sup>Decay corrected.

### 3.2 | [<sup>68</sup>Ga]Ga-PSMA-11

A data set of 531 batch records consisting of 190 manual and 341 automated synthesis were analysed (Table 3). For the automation of the PSMA-11 radiolabelling, the experiences obtained from the [<sup>68</sup>Ga]Ga-DOTA-TOC synthesis were directly implemented. Therefore, only a comparison of the optimized manual and automated procedures is possible.

Such as for [<sup>68</sup>Ga]Ga-DOTA-TOC, the starting activities are in the same range for both automated and manual synthesis.

Both methods show significant differences. Conducting the manual method leads to the final product within an average of  $21:50 \pm 6:22$  minutes compared with  $15:07 \pm 4:12$  minutes with the automated method (Table 3).

Comparing the activity yields, significantly lower yields of  $66.8 \pm 14.5\%$  (AY) for the manual method opposite to  $83.3 \pm 16.0\%$  (AY) for the automated process reflected this prolonged synthesis duration. The RCY show similar results  $83.0 \pm 16.4\%$  for the manual method opposite to  $92.1 \pm 18.6\%$  for the automated process. Admittedly, the data set includes data from all batches produced independently from the cause of failure rate. Again revision of the data set leads to the exclusion of overall 70 failed syntheses (25 manual; 45 GAIA), which means an exclusion of 13.2% (13.2% manual, 13.2% GAIA) failed synthesis induced by nonprocess-related causes. None of these batches failed because of the process used. Non-process-related causes observed were low peptide quality, incorrect or poor preparation of the synthesis by the operator, aborted connection pc-device, and a damaged generator. There are 0 failed synthesis in the revised data set for both methods.

**TABLE 4** Consequences of GAIA and manual yields using the example of [<sup>68</sup>Ga]Ga-PSMA-11 for comparison assuming 70-kg-heavy patients, administering 2-MBq/kg bodyweight and 30 minutes between final release of the product and every injection

Patient No.	Injection Time, min	Activity Required, MBq	Activity Provided, MBq	
			GAIA	Manual
			1000	
	0		880	705
1	30	190	690	515
2	60	259	431	256
3	90	352	79	—

Notes. As shown in Table 4 for the manual method, one less patient dose would be available resulting in a need for another synthesis including all consequences (eg, radiation exposure for operator, costs for material, and contaminated waste materials).

As shown in Table 3 for both processes, the mean values increase while the standard deviation drops significantly after revision, from  $66.8 \pm 14.5\%$  to  $70.5 \pm 8.6\%$  (AY) and  $83.0 \pm 16.4\%$  to  $87.1 \pm 9.4\%$  (RCY) for the manual and  $83.3 \pm 16.0\%$  to  $88.0 \pm 7.3\%$  (AY) and  $92.1 \pm 18.6\%$  to  $97.3 \pm 9.8\%$  (RCY) for the automated synthesis.

For the automated synthesis of [<sup>68</sup>Ga]Ga-PSMA-11, the product was obtained within  $14:49 \pm 2:41$  minutes on average, which decreases the time needed by ~30%. After validation of the process, it usually runs without further disturbances, so stable time values are as expected. The radiochemical yield is  $97.3 \pm 9.8\%$  on average, which is an increase of ~11%.

**TABLE 3** Comparison of GAIA and manual synthesis data for [<sup>68</sup>Ga]Ga-PSMA-11 including and excluding nonprocess-related failed productions

		Complete Data Set				Revised Data Set			
		GAIA		Manual		GAIA		Manual	
		M	SD	M	SD	M	SD	M	SD
Process	n	341		190		296		165	
	A <sub>Start</sub> , GBq	1.289	0.233	1.368	0.353	1.302	0.215	1.387	0.334
	A <sub>Product</sub> , GBq	1.039	0.265	0.914	0.284	1.097	0.199	0.970	0.230
	t <sub>Process</sub> , mm:ss	15:07	4:12	21:50	6:22	14:49	2:41	20:48	5:19
	AY, % <sup>a</sup>	83.3	16.0	66.8	14.5	88.0	7.3	70.5	8.6
	RCY, % <sup>b</sup>	92.1	18.6	83.0	16.4	97.3	9.8	87.1	9.4

<sup>a</sup>Nondecay corrected.

<sup>b</sup>Decay corrected.

### 3.3 | Quality control

Quality control was performed according to the specifications given by the European Pharmacopoeia (Ph Eur) in the monograph for [ $^{68}\text{Ga}$ ]Ga-DOTA-TOC14. For both production methods as well as for both tracers the specifications were always met. Radiochemical purity of the final products was determined with >99% on average independent from the production route.

### 3.4 | Statistical analysis

The comparison of automated syntheses [ $^{68}\text{Ga}$ ]Ga-PSMA-11 with manual syntheses [ $^{68}\text{Ga}$ ]Ga-PSMA-11 showed a  $P$  value of  $P < .001$ . For the comparison of automated syntheses [ $^{68}\text{Ga}$ ]Ga-DOTA-TOC with manual syntheses [ $^{68}\text{Ga}$ ]Ga-DOTA-TOC showed a  $P$  value of  $P < .001$ . Both showed, by conventional criteria, a difference, which is considered statistically significant.

## 4 | DISCUSSION

In the present study, synthesis data from the 2015 to 2017 period of clinical routine were analysed and compared; 837 batch records were considered in the complete data set. In order only to compare the performance of both processes the complete data set was revised as described in the results.

During this period a total of 10  $^{68}\text{Ge}/^{68}\text{Ga}$  generators, with nominal  $^{68}\text{Ga}$  activity of 1.85 GBq at calibration time, were used. The generators were replaced every 4 months to ensure batch activities higher than 750 MBq per batch, which adds up to three to four patients per batch. Accordingly, the average starting activities are in the same range independently from tracer or synthesis method but with high standard deviations.

Considering generator physics, the validity of activity-related data (eg, product activity and molar activity) and corresponding standard deviations have to be handled with care. It explains the high standard deviation of the starting activities and partially the high standard deviation of the product activities. For this reason, the yields are of greater significance, both AY and RCY. These values and corresponding standard deviations describe the suitability of a process for a particular radiolabelling reaction.

The average difference in process duration between both methods is 4.3 minutes ([ $^{68}\text{Ga}$ ]Ga-DOTA-TOC) and 6.7 minutes ([ $^{68}\text{Ga}$ ]Ga-PSMA-11) considering the respective complete data set as well as 4.0 minutes ([ $^{68}\text{Ga}$ ]Ga-DOTA-TOC) and 6.0 minutes ([ $^{68}\text{Ga}$ ]Ga-PSMA-11) for

the revised data set. This is equivalent to a loss of ~4.0% of  $^{68}\text{Ga}$  activity of [ $^{68}\text{Ga}$ ]Ga-DOTA-TOC respectively ~6.0% [ $^{68}\text{Ga}$ ]Ga-PSMA-11 because of the longer process duration of the manual method. AY reflects this result, which is significantly lower for the manual method compared with the automated process. This difference would increase even if the start of synthesis (SoS) for both methods would be the same, which was not possible because of the setup of the manual radiolabelling. For the automated process, SoS, the time point of activity measurement of  $^{68}\text{Ga}$  activity trapped on the SCX (before postprocessing) was used. As for the starting activity and SoS, the time point of activity measurement of  $^{68}\text{Ga}$  activity, eluted from the SCX cartridge (after postprocessing), was used. One reason for this is radiation protection for the operator. To measure the activity trapped on the SCX, as the module automatically does, manual removal of the SCX would be necessary. This manual intervention would increase the radiation dose of the operator. The time point of activity measurement at SoS was used for decay correction. Therefore, the process duration excludes the  $^{68}\text{Ga}$  eluate postprocessing for the manual method, while it is included for the automated synthesis. The calculated average time difference and loss of  $^{68}\text{Ga}$  activity is accordingly underestimated. For both methods, the loss of gallium-68 due to retention on the SCX cartridge is less than 1% of the starting activity.

Additionally, since PSMA-11 has to be taken into account, the automated synthesis includes a subsequent  $\text{C}_{18}$  purification step, while the manual process works without  $\text{C}_{18}$  purification.

Nevertheless, synthesis time observed for the manual method is longer than for the automated process. The synthesis setup of the manual method explains this curious result. While the module system measures radioactivity online, these measurements require manual intervention operation.

Additionally, the manual method established at the institution contains a pH measurement to ensure radiolabelling with optimum results after the addition of the eluate to the reaction mixture, followed by manual closing and crimping of the reaction vial. Although this step is not necessary, it is included and executed as described in the documented procedure. The manual pH adjustment in the case of too high aberrations leads to prolonged mean synthesis duration compared with the automated method where an intervention for pH measurement is not possible. While the automated process has defined periods for the entire process steps, the synthesis duration of the manual method depends on the operator's skills and device setup. For example, factors are speed and routine of the operator or distance and reachability of the dose calibrator in relation to the



working area. Accordingly, for the manual method a higher standard deviation is anticipated.

As for the end of synthesis, the time point of measuring the product activity in the final formulation was defined. For both processes, measurement of product activity is performed manually after withdrawal of the quality control sample. As the module cannot measure the final product activity automatically and to eliminate deviations due to the withdrawal of the quality control sample the product activity was not used to determine AY and RCY. Calculation of AY and RCY are based on the activity measured after trapping and elution on the C<sub>18</sub> cartridge in relation to the starting activity. The manual synthesis of PSMA-11 is an exception. Here, the calculation is based on the activity values for product and start activity. This proceeding reduces the influence of the manual withdrawal of the quality control sample. For, eg, the automated process stops after final formulation and the operator has to disconnect the product vial, retrieve the product sample and transfer the vial to the measurement chamber manually. For [<sup>68</sup>Ga]Ga-DOTA-TOC (revised data set), the process duration from SoS until end of final formulation was found to be  $16.17 \pm 0.42$  minutes, while the average duration of removal and measurement of the product vial needs  $2.40 \pm 5.63$  minutes.

The prolonged synthesis duration found for the manual process is also reflected by the AY, which is defined as the nondecay-corrected amount of radioactive product (expressed in Bq) obtained from a starting amount of radioactivity. AY is significantly dependent on process duration, losses of radioactivity in the system (eg, tubing, needles, syringes, and vials) and the radiolabelling yield of the reaction. The average difference found between both methods was 23.2% ([<sup>68</sup>Ga]Ga-DOTA-TOC) and 16.5% ([<sup>68</sup>Ga]Ga-PSMA-11) considering the respective complete data set as well as 18.7% ([<sup>68</sup>Ga]Ga-DOTA-TOC) and 17.5% ([<sup>68</sup>Ga]Ga-PSMA-11) for the revised data set. For both radiopharmaceuticals, the automated process is significantly better than the manual method, although the automated process produces more contaminated waste material than the manual synthesis.

Within the evaluation period, two adjustments of the automated [<sup>68</sup>Ga]Ga-DOTA-TOC process were implemented in clinical routine production. As these changes should improve the process, the revised data set was analysed with regard to these adjustments.

First, Phenomenex SCX was exchanged with the original provided SCX cartridge (Agilent SCX). This adjustment was necessary because of the use of iThemba <sup>68</sup>Ge/<sup>68</sup>Ga generators in clinical routine. In a detailed screening with different generators and cartridges, this cartridge showed a better performance in combination

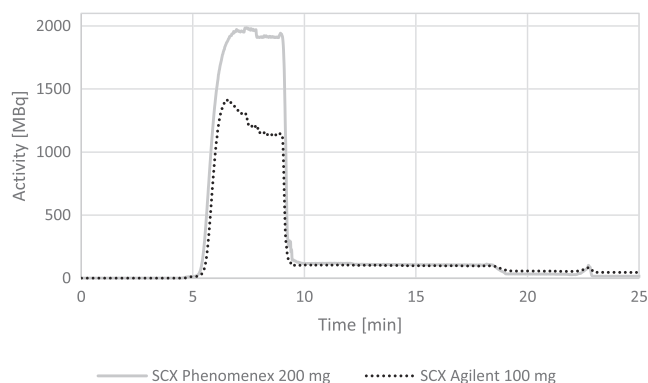
with the iThemba generator. As the iThemba <sup>68</sup>Ge/<sup>68</sup>Ga generator is eluted with 0.6 N HCl, the capacity of the original Agilent SCX cartridge was exhausted. This results in an unwanted premature wash-off of gallium-68 from the SCX during generator elution, leading to reduced starting activity as shown in Figure 4.

Exchange of the SCX resulted in a significant increase of AY (average difference of 16.5%) and RCY (average difference 22.6%) because of distinct reduced process duration (average difference 7.87 min). Additionally, the reliability and reproducibility of the process increased as shown by the almost-halved standard deviations for AY and RCY. Incomplete trapping of the SCX cartridge also influenced the eluted activities, leading to reduced yields.

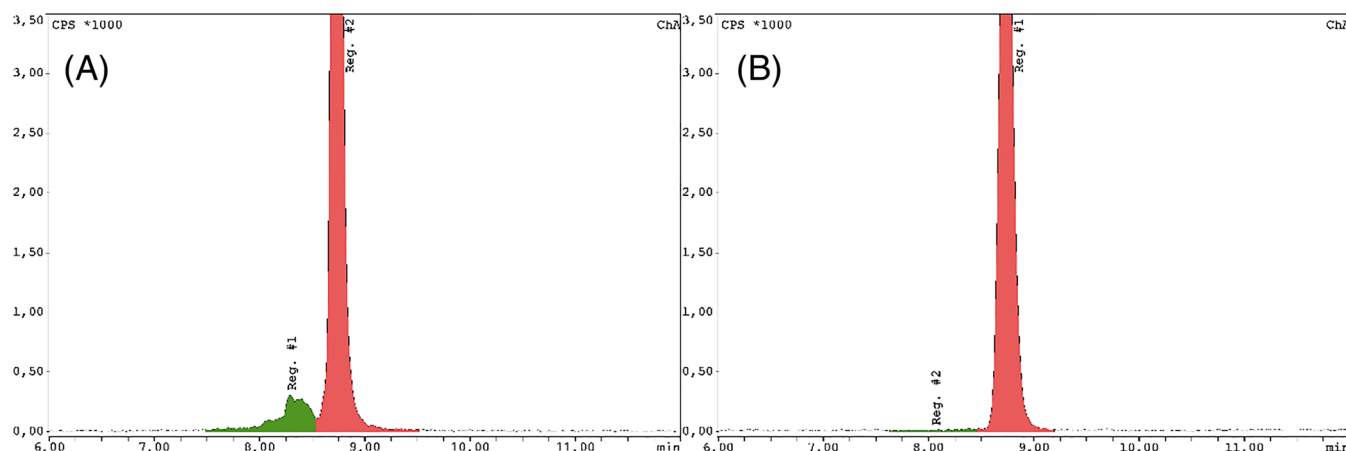
Second, additional 5-vol% ethanol was added to the reaction mixture mainly to inhibit radiolysis,<sup>19-21</sup> the effect of improving radiolabelling efficacy as described in literature<sup>18,22,23</sup> was just secondary as the process was not changed in terms of temperature or heating time. As expected, there are no significant differences in process duration (0.72 min) and RCY (0.10%). In addition, the standard deviations decrease again. Nevertheless, inhibition of radiolysis is effective as determined by HPLC Figure 5.

As the two adjustments were directly adopted to the PSMA-11 process, no data exists for the use of the original SCX or without additional ethanol.

Both automated methods are able to provide the entire radiopharmaceutical with a high reproducibility, and AY and RCY are significantly superior to the manual methods. With average AY higher than 75% and average RCY higher than 90%, the automated methods are very well designed for the synthesis of <sup>68</sup>Ga



**FIGURE 4** Trapping of gallium-68 during elution of an iThemba <sup>68</sup>Ge/<sup>68</sup>Ga generator using 0.6 N HCl on the Phenomenex SCX and the original Agilent SCX during the automated process. The generator was used under the same conditions (first elution of the day, last elution longer than 12 h ago)



**FIGURE 5** RadioHPLC chromatograms of [ $^{68}\text{Ga}$ ]Ga-DOTA-TOC representing a synthesis without (A) and with additional ethanol (B) in the reaction mixture. Sample collection took place directly after final formulation. In region 1 ( $8.12 \pm 0.13$  min), radiolysis by-products can be found. The signal at  $8.66 \pm 0.08$  minutes in region 2 indicates the product [ $^{68}\text{Ga}$ ]Ga-DOTA-TOC

radiopharmaceuticals. When compared with the manual procedure, the automated process provides higher yields, higher reliability, and lower radiation doses to the operator, although it also leads to more contaminated waste material. The international Commission on Radiological Protection proposed principles of radiological protection<sup>24</sup>:

- The Principle of Justification: Any decision that alters the radiation exposure situation should do more good than harm.
- The Principle of Optimization of Protection: The likelihood of incurring exposure, the number of people exposed, and the magnitude of their individual doses should all be kept as low as reasonably achievable, taking into account economic and societal factors.
- The Principle of Application of Dose Limits: The total dose to any individual from regulated sources in planned exposure situations other than medical exposure of patients should not exceed the appropriate limits specified by the Commission.

Assuming that the principles one and three will be observed, it would be logically consistent to switch from the manual method to the much more stable automated procedure with regard to radiation protection of the operator. As example, for PSMA-11, based on 1000 MBq starting activity, the automated synthesis would yield 880-MBq final product, while the manual method only yields 705 MBq (difference of 175 MBq). An amount, which could be required for an additional 70-kg patient administering 2.0-MBq/kg bodyweight.

## 5 | CONCLUSIONS

The evaluation of these 837's clinical routine batch records (a set of 723 revised data) revealed that the automated procedure established utilizing a cassette module is superior to the traditional established manual method for routine production. This is true for a complete labeling process duration, AY, and RCY.

Although these systems and their accessories are relatively expensive in acquisition, they have several advantages over the manual mode as well as the kit-type radiolabelling method:

- Suitability for a wide range of tracers and radionuclides
- Improves practicality of harmonized and standardized multicentre clinical trials<sup>21</sup>
- Reduced amounts of precursor possible (eg,  $2.58 \pm 0.42$ - $\mu\text{g}$  PSMA-11 for automated process compared with 25- $\mu\text{g}$  PSMA-11 in the cold kit (eg, ANMI SA, Belgium))
- Radiation protection
- Reduced risk of cross contamination and viable/nonviable particles due to the use of disposable, sterile reagent kits, and manifolds

Besides that, main advantages of automation are its higher reliability, better reproducibility, and time saving. This is also supported by the findings of other studies investigating automated radiolabelling.<sup>17,25,26</sup>

The *t* tests showed a significant difference between manual and automated syntheses. For both [ $^{68}\text{Ga}$ ]Ga-PSMA-11 and [ $^{68}\text{Ga}$ ]Ga-DOTA-TOC, the automated synthesis mode is superior to the manual synthesis.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Conceptualization, EE; Data Curation, EE and MM; Formal Analysis, EE and MM; Investigation, EE and MM; Methodology, EE; Project Administration, EE; Writing—Original Draft Preparation, MM; Writing—Review & Editing, EE; Supervision, EE

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